IZABEL CRISTINA ALVES BATISTA

Unveiling the hidden threat: The interactions between soybean and Oomycetes, with a focus on *Pythium*-like and *Phytophthora sojae*

Tese apresentada à Universidade Federal de Viçosa como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Doctor Scientiae*.

Orientador: Eduardo Seiti Gomide Mizubuti

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Izabel Cristina Alves Batista
Autora

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Eduardo Seiti Gomide Mizubuti
Orientador

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BIOGRAFIA

Izabel Cristina Alves Batista nasceu em Castanhal, Pará, em 29 de março de 1993, filha de Francisca Alves Batista. Iniciou sua trajetória acadêmica em março de 2010 ao ingressar no curso de Agronomia na Universidade Federal Rural da Amazônia - UFRA, em Belém, Pará. Durante os anos de 2013 e 2014, participou de um programa de intercâmbio sanduíche na University of Manitoba - UofM, Canadá. Formou-se em Agronomia em julho de 2017, em agosto do mesmo ano, deu início ao mestrado em Fitopatologia na Universidade Federal de Viçosa - UFV. Em 2020, iniciou o doutorado em Fitopatologia na UFV. Desde outubro de 2023, é pesquisadora na Bayer Crop Science, atuando como responsável técnica na estação quarentenária em Petrolina-PE.

RESUMO

BATISTA, Izabel Cristina Alves, D.Sc., Universidade Federal de Viçosa, novembro de 2023. **Revelando uma ameaça oculta: As interações entre a soja e os oomicetos, com foco nos organismos semelhantes a *Pythium* e *Phytophthora sojae***. Orientador: Eduardo Seiti Gomide Mizubuti.

Este estudo aborda oomicetos patogênicos habitantes do solo que podem afetar a produção de soja devido a doenças causadas por estes micro-organismos como a podridão de sementes e raízes e tombamento de plântulas. No primeiro capítulo, diversas espécies de *Pythium*, *Globisporangium*, *Phytophthora*, *Phytopythium* e *Aphanomyces*, foram isoladas de áreas afetadas na região Centro-Sul do Brasil. A identificação foi realizada por meio de análises filogenéticas, destacando a prevalência de *Pythium myriotylum*, *Globisporangium irregulare* e *Pythium deliense*, além de *Phytophthora sojae*, a espécie de oomiceto já conhecida por causar a podridão da raiz e da haste (PRH). A patogenicidade de isolados semelhantes a *Pythium* foi avaliada, com destaque para *G. ultimum*, *G. ultimum* var. *sporangiiferum*, *G. irregulare* e *P. myriotylum*, que apresentaram o maior Índice de Severidade da Doença em ensaios de semente. Além disso, a sensibilidade dessas espécies a vários oomicidas foi avaliada, sendo mefenoxam particularmente eficaz. No segundo capítulo, o estudo se concentra na dinâmica de *P. sojae*, o oomiceto que causa a PRH. A pesquisa caracteriza 40 isolados de uma região com alta incidência no Brasil, identificando 28 patótipos. Uma mudança significativa na diversidade de patótipos na última década destaca a importância do monitoramento das populações de *P. sojae* para o uso estratégico de genes de resistência em programas de melhoramento de soja. No terceiro capítulo, a pesquisa explorou a diversidade genética e a evolução temporal das populações de *P. sojae* em regiões produtoras de soja no Brasil ao longo de uma década. Utilizando marcadores microssatélites e determinando patótipos com base nas respostas a linhagens diferenciais de soja, foram identificados 37 patótipos únicos em 2023, indicando um aumento na diversidade em comparação com 2013. Essas descobertas contribuem para uma compreensão mais aprofundada da diversidade e patogenicidade de oomicetos na soja, fornecendo informações valiosas para o manejo de doenças. Elas destacam a importância da resistência genética e a necessidade de abordagens estratégicas no desenvolvimento de novas cultivares com genes de resistência.

Palavras-chave: Patogenicidade. Resistência. Soja. Podridão da raiz. Genética populacional.

ABSTRACT

BATISTA, Izabel Cristina Alves, D.Sc., Universidade Federal de Viçosa, November, 2023. **Unveiling the hidden threat: The interactions between soybean and oomycetes, with a focus on *Pythium*-like and *Phytophthora sojae***. Adviser: Eduardo Seiti Gomide Mizubuti.

This study addresses important aspects of soilborne pathogenic oomycetes that can impact soybean production due to diseases such as seed and root rot, as well as seedling damping-off. In the first chapter, various species of *Pythium*, *Globisporangium*, *Phytophthora*, *Phytopythium*, and *Aphanomyces*, were isolated from affected areas in the Central-South region of Brazil. Identification was conducted through multigene phylogenetic analyses, emphasizing the prevalence of *Pythium myriotylum*, *Globisporangium irregulare*, and *Pythium deliense*, in addition to *Phytophthora sojae*, the oomycete species already known to cause Soybean Root and Stem Rot (SRSR). The pathogenicity of *Pythium*-like isolates was assessed, with an emphasis on *G. ultimum*, *G. ultimum* var. *sporangiiferum*, *G. irregulare*, and *P. myriotylum*, which exhibited the highest Disease Severity Index in seed assays. Additionally, the sensitivity of these species to various oomycides was assessed, with mefenoxam proving to be particularly effective. In the second chapter, the study focuses on the dynamics of *P. sojae*, the oomycete that causes SRSR. The research characterizes 40 isolates from a high-incidence region in Brazil, identifying 28 pathotypes using soybean differentials. A significant shift in pathotype diversity over the past decade highlights the importance of monitoring *P. sojae* populations for strategic deployment of resistance genes in soybean breeding programs. In the third chapter, the research explored the genetic diversity and temporal evolution of *P. sojae* populations in Brazilian soybean-producing regions over a decade. Using microsatellite markers and determining pathotypes based on responses to soybean differential lines carrying resistance genes, 37 unique pathotypes were identified in 2023, indicating an increase in diversity compared to 2013. These findings contribute to a deeper understanding of oomycete diversity and pathogenicity in soybean, providing valuable information for disease management and underscoring the importance of genetic resistance and the need for strategic approaches in developing new cultivars with resistance genes.

Keywords: Pathogenicity. Resistance. Soybean. Root rot. Population genetics**.**

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General context

Soybean is one of the most valued crops and has been designated as essential for human and animal diet, and biodiesel production (Guriqbal and Shivakumar 2010). In 2021, Brazil produced 134.9 million tons, making it the world's largest soybean producer, followed by the United States of America and Argentina (Faostat 2023). However, soybean production faces significant challenges due to diseases caused by various pathogens, including oomycetes (Guriqbal and Shivakumar 2010).

Soybean diseases caused by species of oomycetes have gained considerable attention worldwide due to high yield losses and the increasing diversity of causal agents of pre-emergence and seedling pathologies. Among these oomycete pathogens, those belonging to the genera *Pythium* (*Globisporangium*), *Phytopythium*, and *Phytophthora* (Rojas et al. 2017; Grijalba et al. 2020; Molin et al. 2021) have been identified as major contributors to soybean health challenges.

Particularly destructive to soybeans is the species *Phytophthora sojae* (Kaufm. & Gerd). This pathogen is known as a host-specific pathogen with the ability to infect and cause plant death at any stage of growth (Tyler and Gijzen 2014). The presence of *P. sojae* in soybean fields poses a serious threat to crop production and has prompted extensive research to understand its epidemiology and devise effective control strategies.

Initially, this thesis aimed to conduct a comprehensive survey of *P. sojae* isolates, the causal agent of soybean root and stem rot (SRSR), with the objective of understanding its genetic diversity and geographical distribution in agricultural areas of interest in Brazil. Additionally, we sought to identify the pathotypes (physiological race) present in these isolates, intending to deepen our understanding of the

interactions between this pathogen and soybeans. However, during the course of our research, we made an unexpected discovery: alongside the *P. sojae* isolates, we obtained a significant number of oomycetes previously described here as "*Pythium*-like", which also demonstrated pathogenicity towards soybeans. This surprising finding prompted a reevaluation of the research scope and objectives.

The present thesis seeks to build upon the established knowledge about *P. sojae* by integrating the study of these "*Pythium*-like" isolates, thus expanding the investigation to encompass a broader spectrum of oomycete pathogens affecting soybean crops. By doing so, our research aims to shed light on the complexities of oomycete pathogen populations and their impact on soybean disease dynamics, ultimately providing valuable insights for the design of comprehensive and effective strategies for disease management in soybean cultivation.

The organization of this thesis reflects the evolution of our discoveries and delves into each investigated aspect. Chapter 1 describes the process of soil sample processing, oomycete isolation, pathogenicity tests, and highlighting the identification of "*Pythium*-like" isolates. Within the set of 370 isolates obtained, isolates belonging to *Pythium* (*Globisporangium*), *Phytopythium*, *Aphanomyces* and *Phytophthora* were pathogenic to soybeans. The findings of this study offer a comprehensive view into the diverse range of oomycetes present in the Center-South region of Brazil. In addition, a sensitivity test with chemical compounds that are registered to be used as seed treatment was assessed to provide essential guidelines for developing effective disease control strategies.

The Chapter 2 addresses the identification of *P. sojae* pathotypes and their comparisons with previous studies to assess the dynamics of the diversity of these pathogens over approximately 10 years. While both the terms “pathotype” and “race”

are used to describe the same concept of physiological race, the preference for the term “pathotype” arises from the need to address the complexity of pathogenic variations more precisely in P. sojae concerning different resistance genes in the host plant.The results of the second chapter demonstrate that genes used 10 years ago, such as 1a, 1c, and 1k are not very effective for the current population of *P. sojae*, which has already overcome the resistance of these genes. It was observed a shift in pathotype diversity and complexity of *P. sojae* in Brazil. The current population of *P. sojae* in the southern region of Brazil presents higher numbers of different pathotypes and of avirulence genes in a given individual. This result demonstrates the importance of monitoring changes in the population of *P. sojae* regarding the dynamics of avirulence genes capable of overcoming resistance genes (Rps).

Chapter 3 expands on the research into *P. sojae* isolates, since it is considered the main etiological agent that causes damping-off in soybeans, by focusing on the population biology of this pathogen. A thorough study of genetic structure and allelic variations among *P. sojae* isolates enhances our understanding of its distribution and evolution in soybean cultivated regions. By utilizing advanced genetic analysis and genotyping methods, this chapter uncovers key insights into the genetic diversity and relationships among *P. sojae* isolates, providing a comprehensive view of the pathogen's population dynamics.

In conclusion, this multidisciplinary approach expands the frontiers of soybean pathology, shedding light on the oomycetes associated with damping-off, their sensitivity to oomicides, as well as an approach to the population dynamics and pathotypes of the *P. sojae* present in Brazil. The findings of this research offer significant contributions to the development of effective disease management strategies, also providing valuable information for breeding programs that aim to

develop soybean cultivars with resistance to root and stem rot caused by oomycetes to ensure the productivity of soybean crops.

References

FAOSTAT. 2023. Food and Agriculture Organization of the United Nations. Available at: Available at: http://faostat.fao.org.

Grijalba, P. E., Martínez, M. C., and Guillin, E. 2020. Pathotype and SSR variation in *Phytophthora sojae* from the Argentinean Pampas. J. Phytopathol. 168:228–243.

Guriqbal, S., and Shivakumar, B. G. 2010. The role of soybean in agriculture. In The soybean: botany, production and uses, Wallingford: CABI. 24–47.

Molin, C., Ribeiro, N. R., Matsumoto, M. N., Fernando Giasson, N., Brollo, J., Zanardo, B., et al. 2021. Damping-off of soybean in southern Brazil can be associated with different species of *Globisporangium* spp. and *Pythium* spp. Plant Pathol. 70:1686–1694.

Rojas, J. A., Jacobs, J. L., Napieralski, S., Karaj, B., Bradley, C. A., Chase, T., et al. 2017. Oomycete species associated with soybean seedlings in North America-part I: Identification and pathogenicity characterization. Phytopathology. 107:280–292.

Tyler, B. M., and Gijzen, M. J. 2014. The *Phytophthora sojae* genome sequence: Foundation for a revolution. Pages 133-157 in: Genomics of Plant-Associated Fungi and Oomycetes: Dicot Pathogens. R. A. Dean, A. Lichens-Park, and C. Kole, eds. Springer, Heidelberg, Germany.

CHAPTER 1 - Sorting oomycete species associated with seed, root and stem rot at early stages of soybean development

I. C. A. Batista[1], A. L. Silva Junior[1], G. C. F. de Melo[1], A. F. Silva[1], and E. S. G. Mizubuti[1†]

[1]Departamento de Fitopatologia, Universidade Federal de Viçosa, MG, 36570-900, Brasil.
†Corresponding author: E.S.G. Mizubuti; E-mail: mizubuti@ufv.br

Abstract

Oomycetes are economically significant soilborne pathogens, which accounts for soybean yield decline worldwide due to seed and root rot and damping-off of soybean plantlets. Several genera and species have been associated with these diseases. In this study, five *Pythium* species (*Pythium angustatum*, *P. aphanidermatum*, *P. deliense*, *P. myriotylum* and *P. oopapilum*), four *Globisporangium* species (*Globisporangium acanthophoron*, *G. irregulare*, *G. ultimum*, and *G. ultimum* var. *sporangiiferum*), one *Phytophthora* species (*Phytophthora sojae*), one *Phytopythium* species (*Phytopythium cucurbitacearum*), and one *Aphanomyces* species (*Aphanomyces cladogamus*) were isolated from severely rotted soybean stems, roots, or baited from soil sampled in yield-decline-affected areas in the Central-South region of Brazil. Identification was carried out through phylogenetic analyses utilizing partial sequences of the ITS and cytochrome oxidase I genes. *Phytophthora sojae*, *Pythium myriotylum*, *Globisporangium irregulare*, and *Pythium deliense* were the most prevalent. During the pathogenicity analyses, *P. sojae* clearly differed from the other species due to its distinct host-pathogen interaction involving gene-for-gene interactions with soybeans. Our primary focus was to investigate the pathogenicity of the *Pythium*-like isolates. *G. ultimum*, *G. ultimum* var. *sporangiiferum*, *G. irregulare*, and *P. myriotylum* resulted in the highest average Disease Severity Index in the seed assay. Furthermore, the sensitivity of *G. irregulare*, *G. ultimum*, *P. aphanidermatum*, *P. deliense*, and *P. myriotylum* to the oomicides oxathiapiprolin, zoxamide, mefenoxam, and cyazofamid was assessed. Most species exhibited sensitivity solely to mefenoxam, with EC_{50} values ranging from 0.70 µg/ml for *P. deliense* to 81.67 µg/ml for *P. myriotylum*. These findings contribute to a better understanding of the diversity and pathogenicity of oomycetes in soybean and their sensitivity to various oomicides, providing valuable insights for soybean disease management.

Keywords: Pathogenicity, *Pythium* Species, Fungicide Sensitivity, Root Rot,

Damping-off.

1. Introduction

Soybean (*Glycine max*), one of the most important agricultural crops worldwide is primarily cultivated for the production of oil and protein. In 2021, the global soybean production was estimated to have reached 371.7 million tons, and Brazil accounted for 134.9 million tons, making it a significant contributor to the world's soybean production (36%) (Faostat 2023). The United States (32%) and Argentina (12%) also played a substantial role in the world soybean production statistics. The three countries together are responsible for 80% of the total production (Faostat 2023). Given the remarkable performance of Brazil in soybean production, it is imperative to gain a comprehensive understanding of the occurrence of diseases that have the potential to jeopardize crop yield.

Many parasitic microorganisms cause diseases in soybean, including oomycetes (Guriqbal and Shivakumar 2010). Oomycetes are fungus-like organisms, known for their ability to cause diseases to several crops (Nowicki et al. 2012, Radmer et al. 2017; Zhang et al. 2021). Among the oomycetes that infect soybeans, *Phytophthora sojae* (Kaufm. & Gerd.) stands out as a destructive pathogen because it is a host specific pathogen that infects and causes plant mortality at any growth stage (Tyler and Gijzen, 2014). Soybean root and stem rot (SRSR) leads to substantial losses in the southern region of Brazil. Symptoms can be seen since the pre-emergence stage, resulting in decreased yields. This disease is more prevalent in areas where soybeans are cultivated annually (Costamilan et al. 1996).

Populations of *P. sojae*, the primary etiological agent of SRSR, have high genetic variability, making disease control a challenging task. The main disease control strategy involves the use of resistance genes (*Rps*) incorporated into commercial cultivars. However, due to the high genetic variability, the search for effective genes capable of preventing pathogen establishment is of major interest for the breeding

programs of several companies. In Brazil, a shift towards higher diversity and complexity of *P. sojae* pathotypes was recently reported (Batista et al. 2023). Currently, 28 pathotypes of *P. sojae* are known to occur in the South region of Brazil (Batista et al. 2023).

Other oomycetes, mainly *Pythium sensu lato*, have been associated with soybean seedlings pathologies in Brazil (Rojas et al. 2017; Grijalba et al. 2021; Molin et al. 2021). Unlike *P. sojae*, *Pythium-like* species that infect soybeans have a wide range of hosts. These species cause damping-off both at pre- and post-seedling emergence. Given the diversity of *Pythium* spp. associated with soybeans, understanding which ones are pathogenic, how they are distributed and what is the frequency with which they occur is essential for making decisions about which targets should be prioritized in disease management.

These oomycetes are able to survive in crop residues, and under favorable conditions can germinate, develop reproductive structures, and subsequently become attracted via chemotaxis generated by root exudates to infect soybean seedlings (Dorrance 2018; Zhang et al. 2019). The increasing diversity of species of oomycetes causing soybean diseases poses a threat to soybean production, and a better understanding of their characteristics and pathogenicity must be made available.

Thus, the objectives of this study were: to identify the oomycete species associated with soybean in the major producing areas in the central-south region of Brazil and to determine their pathogenicity; to assess the severity of the disease, focusing on the associated pythiaceous species; and to evaluate the sensitivity of pathogenic isolates to different oomicides. By gaining insights into the specific oomycete species affecting soybeans, this research aims to contribute to the development of effective disease management strategies of soybean cultivation.

Furthermore, the generated knowledge will aid in making decisions regarding the protection of soybean crops, benefiting both farmers and the agricultural industry as a whole.

2. Materials and methods

2.1. Collection of soil samples

Soybean-producing fields distributed in five states of the central-south region of Brazil, Mato Grosso do Sul, Paraná, Rio Grande do Sul, São Paulo, and Santa Catarina, were selected and visited to collect soil samples. Whenever suspicious infected soybean plants were spotted in a field, this material was collected. The selected fields are from the soybean-producing edaphoclimatic macro-regions 1 and 2 (SMR) in Brazil. The country is categorized into five soybean macro-regions (SMR) and 20 edaphoclimatic regions (ECRs), representing geographical areas with comparable soil and climate conditions essential for successful soybean cultivation (Kaster and Farias 2012). Our emphasis was on sampling regions with documented instances of disease or suspected disease occurrences (Figure 1).

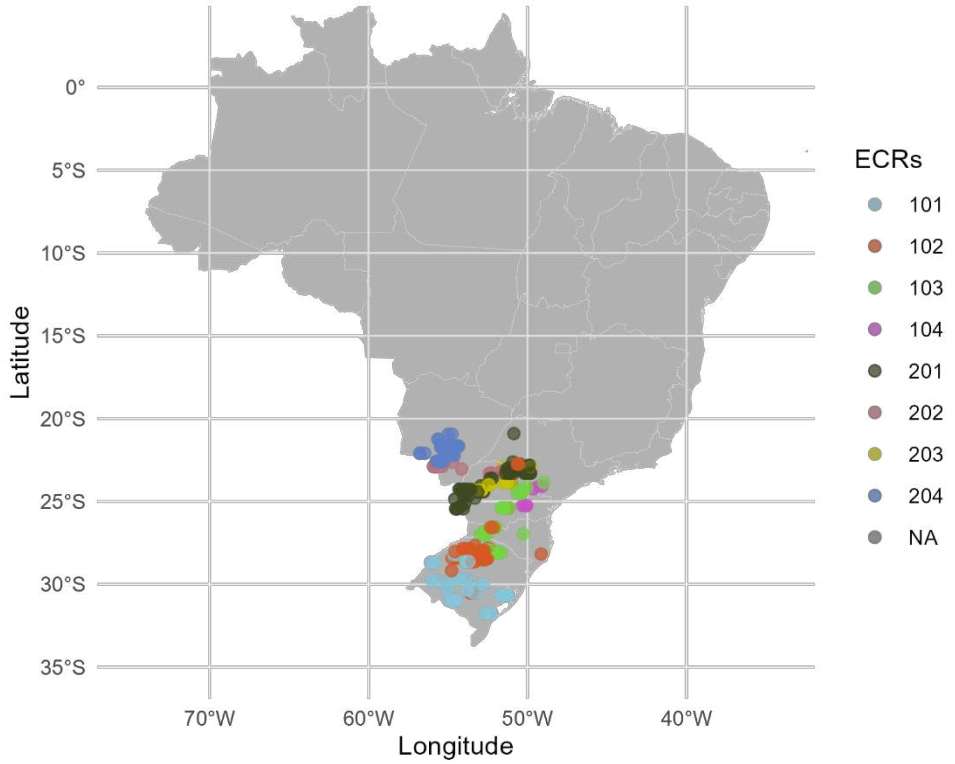


Figure 1. Map of Brazil showing sampled fields in different soybean-producing edaphoclimatic regions (ECRs). In the soybean macro-region 1 and 2 fields located in five states were sampled.

Soil and plant samples were collected from areas where damping-off and root rot caused by oomycetes were reported along three soybean crop seasons: 2020/21, 2021/22, and 2022/23. For the soil samples, a zigzag pattern was followed, taking 10 to 15 subsamples to amount to a total of 1500 g of soil in each area. Plant sampling, on the other hand, was specifically conducted on plants showing symptoms of suspected disease observed in the field. In total 21 plants and 488 soil samples were collected from areas with reported occurrence of damping-off and root rot and also from areas of suspected disease occurrence. From each sample, pathogen isolation was attempted at least twice. In both attempts, two isolation techniques were employed. The methods were as follows.

2.2. Oomycete isolation

The processing of soil samples and isolation of oomycetes were as previously described (Batista et al. 2023). Briefly, two methodologies were employed: soybean leaf baits and a bioassay using seedlings of a susceptible cultivar ('Williams') grown in 0.5 L of soil sample (Supplementary Figure 1). Both methodologies were executed without any prior measures for oospore germination.

For the oomycete isolation, a modified selective oomycete medium was employed, which consisted of the following components: PBNIC, supplemented with 500 mL of carrot juice or V8 juice, 0.05 g of β-sitosterol, 0.005 g of benomyl, 0.054 g of PCNB, 0.1 g of neomycin sulfate, 0.01 g of chloramphenicol, 0.04 g of iprodione, and 15 g of agar dissolved in 1000 mL of distilled water (Dorrance et al. 2008).

Following successful isolation, the isolates were maintained on carrot-agar medium (CA). This CA was prepared by filtering the concentrated juice of 50 g of blended carrots through eight layers of voile fabric. Subsequently, 15 g of agar was added and the volume was adjusted to 1 liter using distilled water. For long-term preservation, the isolates were stored on CA plugs at 8°C with a 10% glycerol solution.

2.3. Oomycetes preliminary screening

The preliminary screening for the identification of oomycetes was performed through a combination of morphological characteristics and a PCR-based molecular analysis. Morphological features, such as the presence of aseptate hyphae and distinctive oomycete reproductive structures (oospores, oogonia, and sporangia) were examined under a microscope. Additionally, a three-step PCR diagnostic approach was employed: First, performing an amplification of the *COI1* gene (cytochrome c oxidase subunit 1) was done with the primers OomCox1Levup and OomCox1Levlo (Robideau et al. 2011), to confirm the presence of oomycetes. The second reaction

targeted the *Ras*-related protein gene *Ypt*1 with the primers YPh1F and YPh2R to identify the presence of *Phytophthora* genus (Schena et al. 2008). The third amplification targeted the transcribed internal spacer region (ITS) using the specific primers PSOJF1 and PSOJR1 (Bienapfl et al. 2011). This step was designed to detect the presence of *Phytophthora sojae*, a species commonly associated with root and stem rot and damping-off in soybean.

Total DNA of the isolates was extracted according to the manufacturer's instructions of the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA), and the PCR reactions were carried out in a T100 thermocycler (Bio-rad, California, USA). For the OomCox reaction, 36 cycles were used, starting with initial denaturation at 95°C for 4 min, followed by cycles of denaturation at 95°C for 40 s, annealing at 50°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The Yph protocol began with initial denaturation at 95°C for 2 min, followed by denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 1 min, repeated for a total of 36 cycles, and final extension at 72°C for 10 min. The PSOJ protocol started with initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 30 s, annealing at 66°C for 30 s, extension at 72°C for 30 s, repeated for a total of 35 cycles, and concluded with a final extension at 72°C for 10 min. After this preliminary screening, the isolates were categorized into two groups: *Phytophthora* and *Pythium*-like isolates.

2.4. Sequencing the pathogenic *Pythium*-like isolates

Among the *Pythium*-like isolates, those pathogenic to soybean were chosen for further analysis through sequencing. Partial sequences of the internal transcribed spacer (ITS) and the COI1 regions were obtained (Robideau et al. 2011). The primer pairs used for PCR amplification of the ITS and COI1 regions were ITS6 and ITS4

(Sapkota and Nicolaisen 2015; Cooke et al. 2000) and OomCox1Levup and OomCox1levlo (Robideau et al. 2011), respectively. The PCR reactions were performed in a thermal cycler with the following conditions: ITS amplification: initial denaturation at 95°C for 1.25 min, followed by 34 cycles of denaturation at 95°C for 35 s, annealing at 62°C for 55 s, extension at 72°C for 50 s, and a final extension at 72°C for 10 min. COI1 amplification: initial denaturation at 95°C for 4 min, followed by 36 cycles of denaturation at 95°C for 40 s, annealing at 50°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were subjected to enzymatic purification using ExoSAP-IT (ThermoFisher Scientific Inc., Massachusetts, USA) following the manufacturer's instructions. The purified PCR products were then submitted for Sanger sequencing.

2.5. Phylogenetic analysis

The quality of chromatograms and assembly of consensus sequences from forward and reverse sequences were performed using SeqAssem (version 07/2008). For the identification of species from the isolates obtained in this study, we compared the ITS and COI1 sequences with the reference sequences (Robideau et al. 2011) (Supplementary Table 1). Sequence alignments were carried out using MUSCLE implemented in MEGA X software (https://www.megasoftware.net).

Concatenated gene alignments were generated using Sequence Matrix v1.8.125. Maximum Parsimony (MP) and Bayesian Inference (BI) trees were constructed for concatenated data. Prior to the phylogenetic analyses, the most appropriate nucleotide substitution model for each locus was selected using MRMODELTEST v.2. Models of nucleotide substitution in the concatenated two-gene trees were HKY + I and GTR + G for COI1 and ITS, respectively. For all trees, BI and ML analyses were conducted on the CIPRES Science Gateway platform using Mr.

Bayes 3.2.7a and RaxML-HPC v.8.2.12, respectively. Phylogenetic trees were visualized using MEGA 10, FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and edited on CorelDraw 2019.

2.6. Pathogenicity of oomycetes

For the *Phytophthora* isolates, the pathogenicity assay was performed using the hypocotyl inoculation method (Hass and Buzzell, 1976; Dorrance et al. 2004). Ten seedlings of the susceptible cv. Williams were inoculated by injecting approximately 0.1 mL of the mycelial slurry of each isolate into the hypocotyl. The pathogenic classification was assigned to isolates that demonstrated the ability to induce mortality in >60% of soybean plants (Supplementary Figure 2C and D).

In the case of *Pythium*-like isolates, two separate assays were conducted (Supplementary Figure 2A and B). In the first assay, a seed pathogenicity test was performed *in vitro*. Mycelium discs were placed on a CA medium, where 10 seeds of soybean cultivar L93-3312 (PI 591516) were previously placed along the edge of the Petri dish (9 mm). Each treatment (isolated strain) was applied to four experimental units, corresponding to one Petri dish containing 10 seeds. The evaluation was conducted seven days after inoculation by counting the number of non-germinated seeds colonized by the Pythiaceae isolate and the number of germinated seeds displaying root lesions.

In the second assay, pathogenicity was evaluated using the sand-cornmeal method (Broders et al. 2007; Kirkpatrick et al. 2006). Briefly, the inoculum was prepared by combining mycelium discs from colonies grown on V8 or CA medium with sand, cornmeal, and distilled water. This mixture was incubated in a growth chamber at 25°C for 10 to 14 days. The resulting sand-corn inoculum was mixed with vermiculite in a 1:4 ratio (v:v) and distributed into 500 mL plastic cups. Ten soybean seeds of cv.

NS 5959 IPRO or Williams were placed on the surface of the infested substrate. Both soybean cultivars were susceptible and had similar symptoms when compared in previous trials (Supplementary Figure 3). Each isolate was applied to four cups, each containing 10 seeds. The assay was conducted in a completely randomized design and the number of germinated seeds was recorded 14 days after sowing.

In both assays, each isolate was examined using four replicates. Due to the extensive number of isolates and replicates, the isolates were divided into small groups, each including a control without the pathogen. To evaluate the disease severity, a rating scale for seed rot was used: 0 = healthy seed germination at 100% without any signs of infection, 1 = germination at 70 to 99% with the formation of lesions on the roots, 2 = germination at 30 to 69% with coalesced lesions, 3 = germination at 0 to 29%, where all seed tissues were colonized, and 4 = no germination occurred, and the seed was colonized (Broders et al. 2007; Rojas et al. 2017). The use of rating scales and data analysis were conducted following the procedures previously described (Rojas et al. 2017) and available in the GitHub repository (https://github.com/Chilverslab/Rojas_Survey_Phytopath_2016).

Using the rating scale, a Disease Severity Index (DSI) was calculated with the formula:

$$DSI = \frac{\Sigma(severityrating \times numberofseedsperrating)}{(totalseeds \times highestseverityratings)} \times 100$$

To fulfill Koch's postulates, the pathogenic isolates were subsequently re-isolated from the infected plant tissues.

2.7. Sensitivity of oomycetes to fungicide

Five pathogenic *Pythium* spp. isolates were subjected to sensitivity tests against four oomicides: oxathiapiprolin, zoxamide, metalaxyl, and cyazofamid. Oxathiapiprolin (Zorvec Enicade; Corteva), zoxamide (Zoxium 240 SC; Gowan Milling, LLC Inc.), cyazofamid (commercial product; Indian Agro Labs), and metalaxyl-M (7042FP; Indian Agro Labs) were dissolved in distilled water to create stock solutions. Sensitivity assays were conducted at various concentrations in a diluted carrot-agar medium (CA). Dilutions were prepared from the stock solution to achieve the desired final concentrations (Table 1) and stored at 4°C in darkness.

Table 1. Oomicides and their concentrations tested in the sensitivity assay.

Treatment name	Product	Active Ingredient	(a.i. g/L)	Dose (µg/mL)	Fungicide Group (FRAC Code)
OXA	Zorvec Enicade	**Oxathiapiprolin**	100	0, 0.00001, 0.0001, 0.005, 0.01, 0.1	OSBPI (49)
ZOX	Zoxium	**Zoxamide**	240	0, 0.0001, 0.005, 0.01, 0.1, 1	Benzamides (22)
CIA	Commercial product	**Cyazofamid**	400	0, 0.1, 1, 10, 50, 100	QiI (21)
MET	7042 FP	**Metalaxyl - M**	350	0, 0.01, 0.1, 1, 10, 100	Phenylamide (4)

The sensitivity assays were conducted using an agar medium method. A 5-mm-diameter plug from a 10-day-old oomycete colony grown on CA was placed in the center of 60-mm-diameter Petri dishes containing CA amended with fungicides as described previously. Non-amended CA was used as the control. The Petri dishes were kept in the dark at 25°C for 10 days, and the colony diameter was measured in two perpendicular directions using digital calipers. Four replicate Petri dishes were used for each treatment, and the assay was conducted twice.

The sensitivity of oomycete isolates was evaluated by determining the half-maximal effective concentration (EC_{50}) for each treatment. The dose-response data

were fitted to a suitable model using the 'mselect()' function from the drcR package (Ritz et al. 2015). The model with the lowest Akaike Information Criterion (AIC) value was selected. The EC_{50} estimates were obtained using the 'estimate_EC50()' function from the EC50estimatorR package (Alves 2020) of the software R.

3. Results

3.1. Sample processing and isolates

A collection of 488 soil samples from the soybean edaphoclimatic macro-regions 1 and 2 was obtained. These samples were sourced from the primary soybean-producing areas within the central-southern region of Brazil. However, it is noteworthy that the plant material samples obtained alongside the soil samples had significantly deteriorated upon arrival at the laboratory. This deterioration compromised the isolation of oomycetes from the plant tissues, resulting in the absence of any oomycete isolates from the plant material. In total, 370 oomycete isolates were recovered from the soil samples collected in 88 municipalities (Supplementary Table 2).

From this set of 370 isolates, an initial screening was performed using a PCR-based molecular analysis to facilitate the classification of the isolates as *Phytophthora* and *Pythium*-like taxa (Figure 2). Specifically, 75 isolates were identified as *Phytophthora sojae*, the causal agent of SRSR, and no other *Phytophthora* species was identified associated with these samples. The remaining 295 isolates were classified as *Pythium*-like organisms (Figure 3). This preliminary screening aimed to delineate the subsequent analysis.

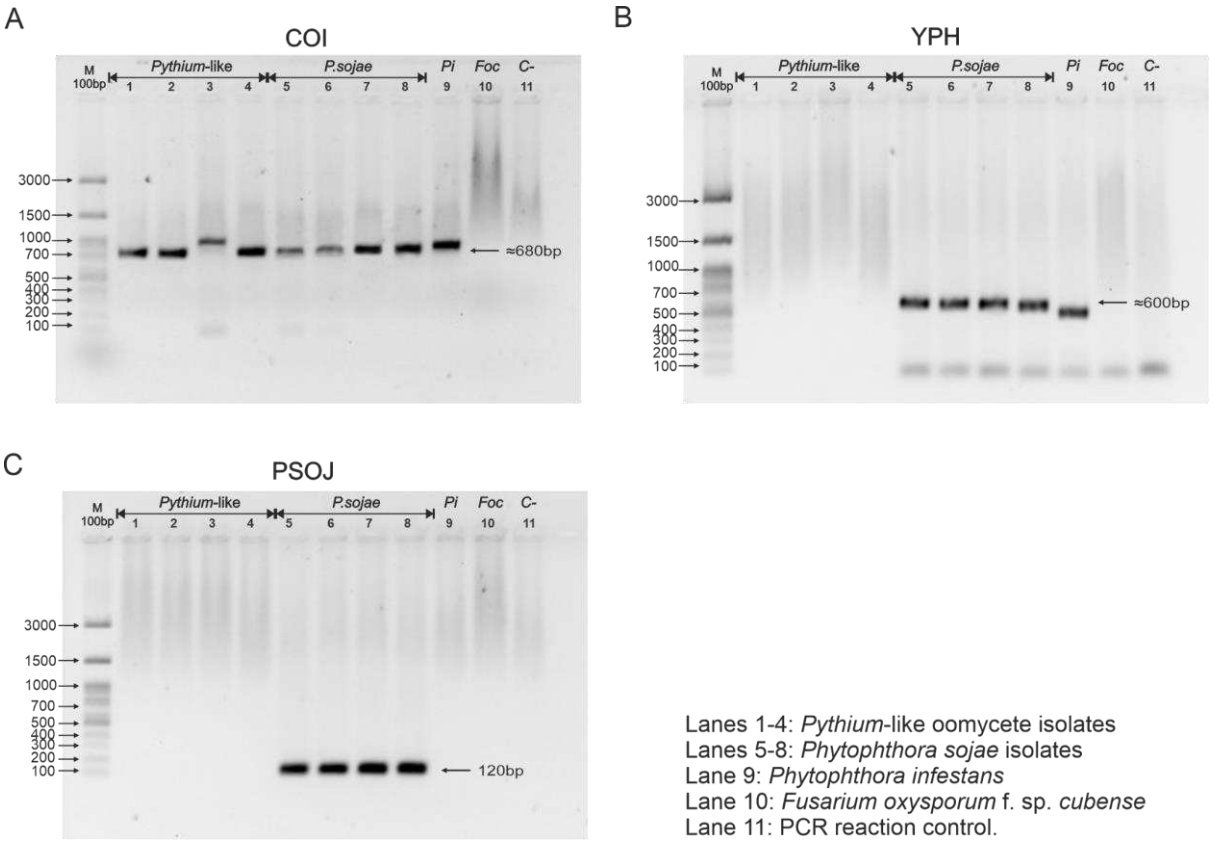


Figure 2. Agarose gel electrophoresis (1% agarose gel) of PCR products obtained for preliminary screening of oomycetes isolates. (A) PCR product results obtained by targeting the COI region. (B) PCR product results from amplification with Yph primers, selectively amplifying *Phytophthora* DNA. (C) PCR product results from amplification with PSOJ primers, where only *Phytophthora sojae* DNA is amplified.

The morphological characteristics of *Phytophthora* differed from those of *Pythium*-like isolates. The branches of *Phytophthora sojae* hyphae tend to be shorter and more frequent, resulting in a more complex and disorganized structure. Additionally, the branches can form wider angles or even clusters of branching, leading to a less directional pattern of growth. On the other hand, the hyphae of most isolates classified as *Pythium*-like tend to be thinner and, in some cases, are defined by the formation of a main hypha with shorter branches emerging from it. Another notable characteristic is that the branches always point in the direction of hyphal growth, giving

them a well-defined orientation. This growth pattern imparts a more linear and organized appearance to *Pythium* hyphae.

The PCR result from the ITS gene region corroborated with the separation of the isolates based on the morphological analysis, confirming that such characteristics are capable of distinguishing the *Phytophthora* and *Pythium*-like taxa.

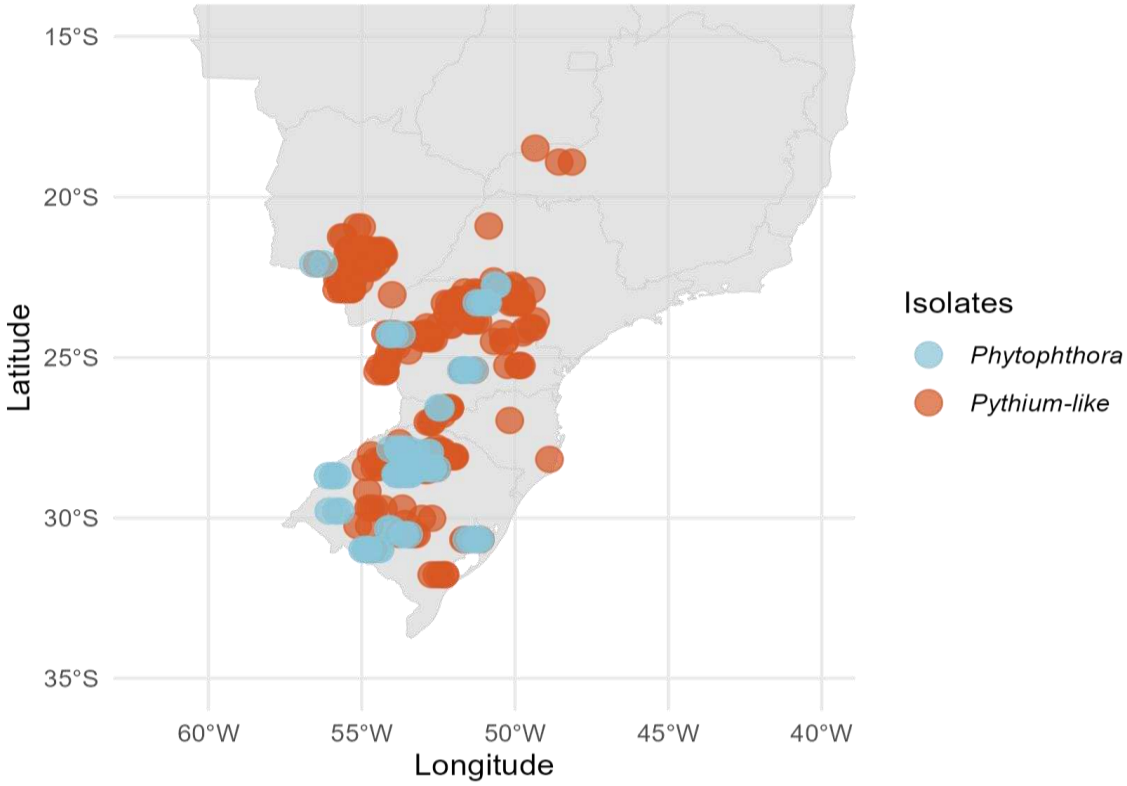
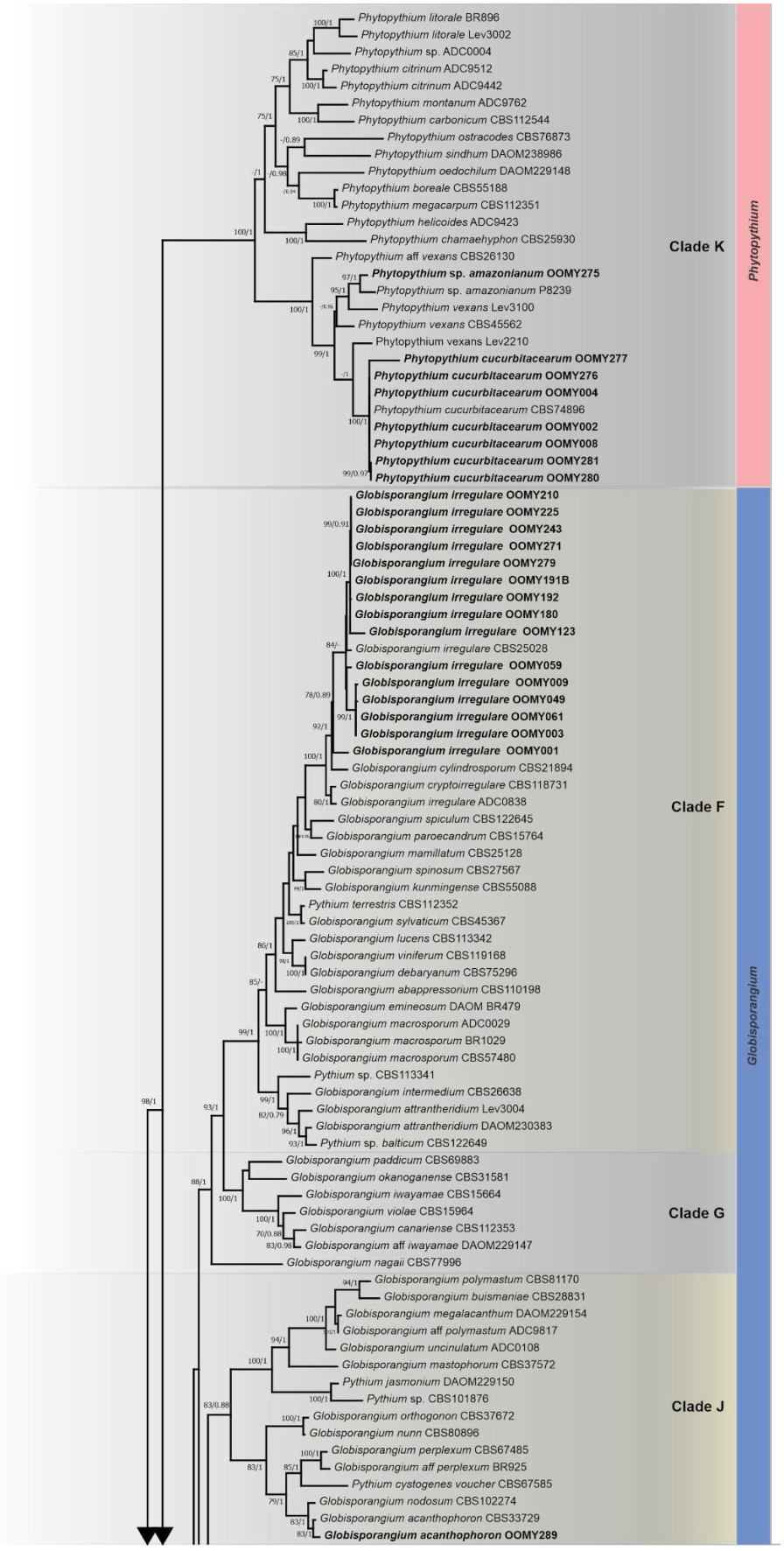


Figure 3. Map of the distribution of isolates of *Phytophthora* spp. and *Pythium*-like recovered from soil samples collected across central-south of Brazil, during the 2020/21, 2021/22, and 2022/23 soybean crop seasons.

3.2. Identification of *Pythium*-like species

In total, 84 *Pythium*-like isolates were subjected to sequencing for species identification. Seventy-four were successfully identified, while 8 failed to produce unambiguous sequences. The ML and BI phylogenetic trees were constructed using both COI and ITS regions. The ITS region comprised 705 bp, while the COI region was 1987 bp. The analyses using the concatenated sequences of ITS and COI included 259 taxa and contained 2692 characters, of which 2063 were parsimony-informative

sites. From the phylogenetic analysis, it was possible to identify 13 different species of oomycetes: one species of *Aphanomyces*, four species of *Globisporangium*, two species of *Phytopythium* and six species of *Pythium* (Figure 4).


Phytopythium litorale BR896
Phytopythium litorale Lev3002
Phytopythium sp. ADC0004
Phytopythium citrinum ADC9512
Phytopythium citrinum ADC9442
Phytopythium montanum ADC9762
Phytopythium carbonicum CBS112544
Phytopythium ostracodes CBS76873
Phytopythium sindhum DAOM238986
Phytopythium oedochilum DAOM229148
Phytopythium boreale CBS55188
Phytopythium megacarpum CBS112351
Phytopythium helicoides ADC9423
Phytopythium chamaehyphon CBS25930
Phytopythium aff vexans CBS26130
Phytopythium sp. amazonianum OOMY275
Phytopythium sp. amazonianum P8239
Phytopythium vexans Lev3100
Phytopythium vexans CBS45562
Phytopythium vexans Lev2210
Phytopythium cucurbitacearum OOMY277
Phytopythium cucurbitacearum OOMY276
Phytopythium cucurbitacearum OOMY004
Phytopythium cucurbitacearum CBS74896
Phytopythium cucurbitacearum OOMY002
Phytopythium cucurbitacearum OOMY008
Phytopythium cucurbitacearum OOMY281
Phytopythium cucurbitacearum OOMY280
Clade K
Phytopythium
Globisporangium irregulare OOMY210
Globisporangium irregulare OOMY225
Globisporangium irregulare OOMY243
Globisporangium irregulare OOMY271
Globisporangium irregulare OOMY279
Globisporangium irregulare OOMY191B
Globisporangium irregulare OOMY192
Globisporangium irregulare OOMY180
Globisporangium irregulare OOMY123
Globisporangium irregulare CBS25028
Globisporangium irregulare OOMY059
Globisporangium irregulare OOMY009
Globisporangium irregulare OOMY049
Globisporangium irregulare OOMY061
Globisporangium irregulare OOMY003
Globisporangium irregulare OOMY001
Globisporangium cylindrosporum CBS21894
Globisporangium cryptoirregulare CBS118731
Globisporangium irregulare ADC0838
Globisporangium spiculum CBS122645
Globisporangium paroecandrum CBS15764
Globisporangium mamillatum CBS25128
Globisporangium spinosum CBS27567
Globisporangium kunmingense CBS55088
Pythium terrestris CBS112352
Globisporangium sylvaticum CBS45367
Globisporangium lucens CBS113342
Globisporangium viniferum CBS119168
Globisporangium debaryanum CBS75296
Globisporangium abappressorium CBS110198
Globisporangium emineosum DAOM BR479
Globisporangium macrosporum ADC0029
Globisporangium macrosporum BR1029
Globisporangium macrosporum CBS57480
Pythium sp. CBS113341
Globisporangium intermedium CBS26638
Globisporangium attrantheridium Lev3004
Globisporangium attrantheridium DAOM230383
Pythium sp. balticum CBS122649
Clade F
Globisporangium paddicum CBS69883
Globisporangium okanoganense CBS31581
Globisporangium iwayamae CBS15664
Globisporangium violae CBS15964
Globisporangium canariense CBS112353
Globisporangium aff iwayamae DAOM229147
Globisporangium nagaii CBS77996
Clade G
Globisporangium polymastum CBS81170
Globisporangium buismaniae CBS28831
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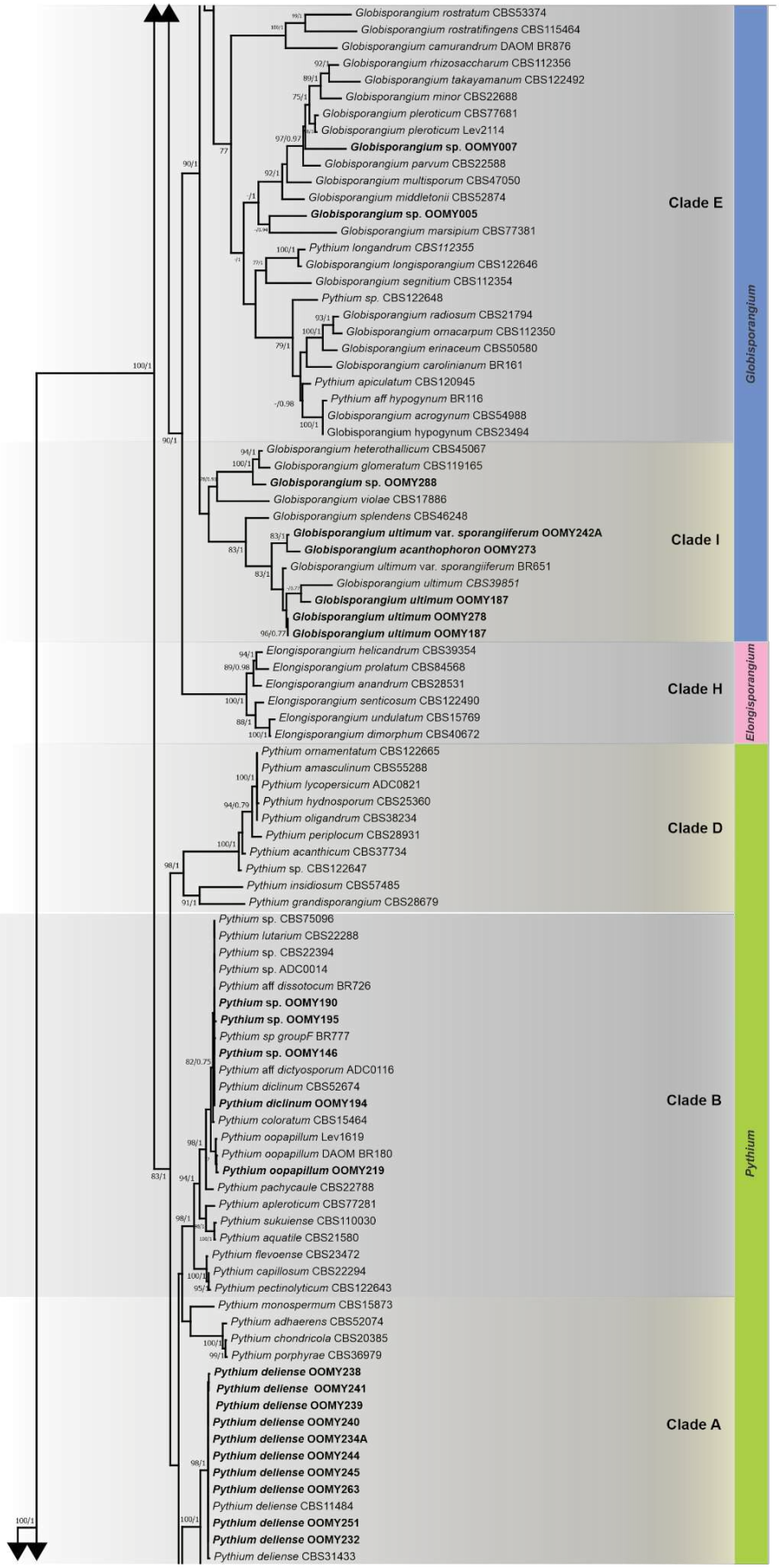
Globisporangium aff polymastum ADC9817
Globisporangium uncinulatum ADC0108
Globisporangium mastophorum CBS37572
Pythium jasmonium DAOM229150
Pythium sp. CBS101876
Globisporangium orthogonon CBS37672
Globisporangium nunn CBS80896
Globisporangium perplexum CBS67485
Globisporangium aff perplexum BR925
Pythium cystogenes voucher CBS67585
Globisporangium nodosum CBS102274
Globisporangium acanthophoron CBS33729
Globisporangium acanthophoron OOMY289
Clade J
Globisporangium


Globisporangium rostratum CBS53374
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Globisporangium camurandrum DAOM BR876
Globisporangium rhizosaccharum CBS112356
Globisporangium takayamanum CBS122492
Globisporangium minor CBS22688
Globisporangium pleroticum CBS77681
Globisporangium pleroticum Lev2114
Globisporangium sp. OOMY007
Globisporangium parvum CBS22588
Globisporangium multisporum CBS47050
Globisporangium middletonii CBS52874
Globisporangium sp. OOMY005
Globisporangium marsipium CBS77381
Pythium longandrum CBS112355
Globisporangium longisporangium CBS122646
Globisporangium segnitium CBS112354
Pythium sp. CBS122648
Globisporangium radiosum CBS21794
Globisporangium ornacarpum CBS112350
Globisporangium erinaceum CBS50580
Globisporangium carolinianum BR161
Pythium apiculatum CBS120945
Pythium aff hypogynum BR116
Globisporangium acrogynum CBS54988
Globisporangium hypogynum CBS23494
Globisporangium heterothallicum CBS45067
Globisporangium glomeratum CBS119165
Globisporangium sp. OOMY288
Globisporangium violae CBS17886
Globisporangium splendens CBS46248
Globisporangium ultimum var. sporangiiferum OOMY242A
Globisporangium acanthophoron OOMY273
Globisporangium ultimum var. sporangiiferum BR651
Globisporangium ultimum CBS39851
Globisporangium ultimum OOMY187
Globisporangium ultimum OOMY278
Globisporangium ultimum OOMY187
Elongisporangium helicandrum CBS39354
Elongisporangium prolatum CBS84568
Elongisporangium anandrum CBS28531
Elongisporangium senticosum CBS122490
Elongisporangium undulatum CBS15769
Elongisporangium dimorphum CBS40672
Pythium ornamentatum CBS122665
Pythium amasculinum CBS55288
Pythium lycopersicum ADC0821
Pythium hydnosporum CBS25360
Pythium oligandrum CBS38234
Pythium periplocum CBS28931
Pythium acanthicum CBS37734
Pythium sp. CBS122647
Pythium insidiosum CBS57485
Pythium grandisporangium CBS28679
Pythium sp. CBS75096
Pythium lutarium CBS22288
Pythium sp. CBS22394
Pythium sp. ADC0014
Pythium aff dissotocum BR726
Pythium sp. OOMY190
Pythium sp. OOMY195
Pythium sp groupF BR777
Pythium sp. OOMY146
Pythium aff dictyosporum ADC0116
Pythium diclinum CBS52674
Pythium diclinum OOMY194
Pythium coloratum CBS15464
Pythium oopapillum Lev1619
Pythium oopapillum DAOM BR180
Pythium oopapillum OOMY219
Pythium pachycaule CBS22788
Pythium apleroticum CBS77281
Pythium sukuiense CBS110030
Pythium aquatile CBS21580
Pythium flevoense CBS23472
Pythium capillosum CBS22294
Pythium pectinolyticum CBS122643
Pythium monospermum CBS15873
Pythium adhaerens CBS52074
Pythium chondricola CBS20385
Pythium porphyrae CBS36979
Pythium deliense OOMY238
Pythium deliense OOMY241
Pythium deliense OOMY239
Pythium deliense OOMY240
Pythium deliense OOMY234A
Pythium deliense OOMY244
Pythium deliense OOMY245
Pythium deliense OOMY263
Pythium deliense CBS11484
Pythium deliense OOMY251
Pythium deliense OOMY232
Pythium deliense CBS31433
Clade E
Clade I
Clade H
Clade D
Clade B
Clade A
Globisporangium
Elongisporangium
Pythium

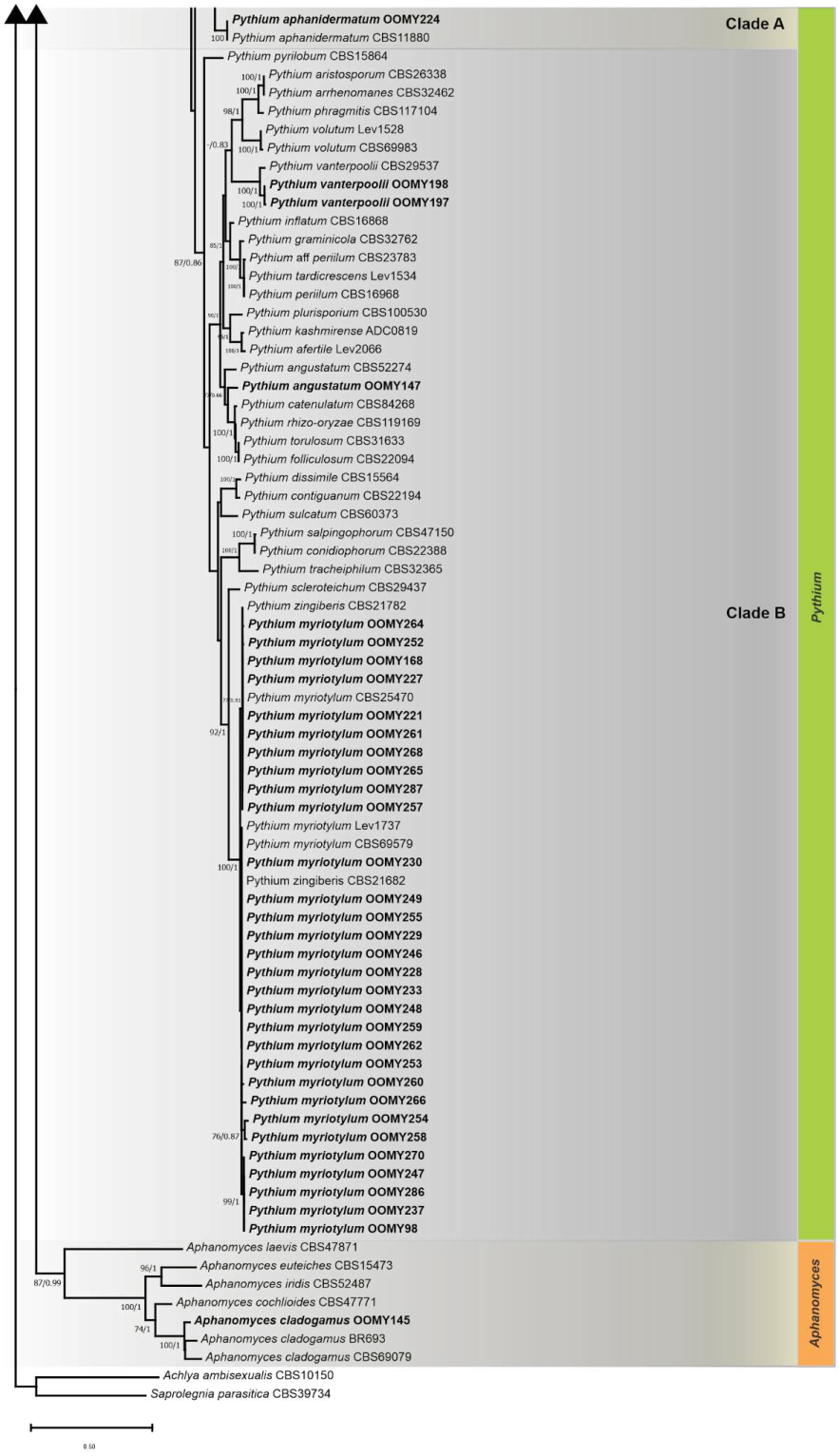

Figure 4. Consensus tree of maximum-likelihood and Bayesian inference phylogenies using a concatenated dataset with ITS and COI sequences from oomycetes. Clades comprising the *Aphanomyces* group and the *Pythium sensu lato* were included. Isolates from this study are in bold. The remaining taxa are reference isolates described in Robideau et al. (2011). Bootstrap support values greater than 70 and posterior probabilities greater than 0.90 are shown on the branches (BS/PP). The

phylogeny is rooted with *Achlya ambisexualis* (CBS 10150) and *Saprolegnia parasitica* (CBS 39734) as outgroup.

The subset of *Pythium*-like isolates displayed a diverse composition: *Aphanomyces cladogamus* (n=1), *Globisporangium acanthophoron* (n=1), *Globisporangium irregulare* (n=15), *Globisporangium ultimum* (n=2), *Globisporangium ultimum* var. *sporangiiferum* (n=2), *Phytopythium* sp. *"amazonianum"* (n=1), *Phytopythium cucurbitacearum* (n=6), *Pythium* angustatum (n=1), *Pythium aphanidermatum* (n=1), *Pythium deliense* (n=10), *Pythium myriotylum* (n=31), *Pythium oopapilum* (n=1) and *Pythium vanterpoolii*. (n=2). Additionally, it is worth noting that three isolates within the *Globisporangium* clade (*Globisporangium* sp., n=3) and four isolates within the *Pythium* clade (*Pythium* sp., n=4) could not could not be identified at the species level since they are members of a clade considered as "a large complex of *Pythium* species Cluster B2a" (Figure 4) (Robideau et al. 2011).

3.3. Oomycetes pathogenic to soybean

Out of 370 isolates, 156 were tested for pathogenicity: 81 isolates were *Pythium*-like and 75 were identified as *Phytophthora sojae*. All 75 *P. sojae* isolates exhibited high levels of virulence towards soybean cv. Williams. Of the 81 isolates initially considered as *Pythium*-like species 72 were pathogenic towards soybean. The remaining 201 isolates of the collection have not been subjected to pathogenicity testing. The DSI varied among the different species of *Pythium*-like *isolates* (Figure 5).

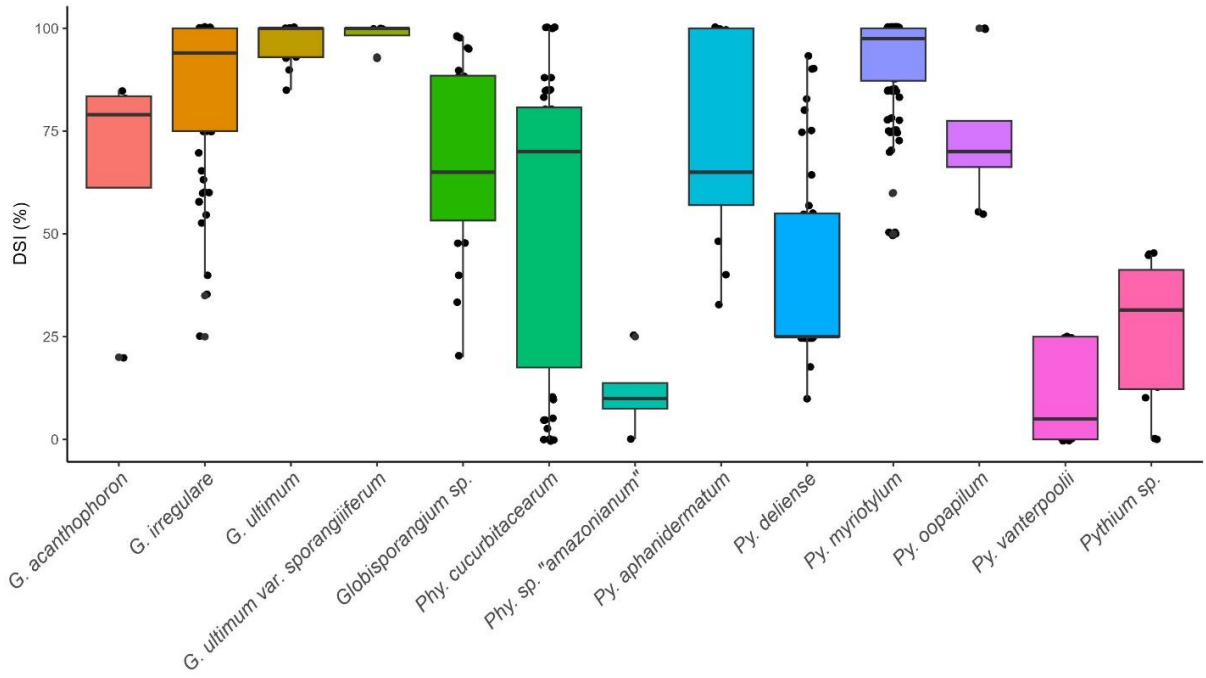

Figure 5. Boxplot of disease severity index (DSI) of different oomycete species on soybean 'Williams'. Each box represents the interquartile range, the line inside indicates the median DSI, and points represents individual data. G. = *Globisporangium*, Phy. = *Phytopythium*, Py. = *Pythium*.

Among the pathogenic isolates, 12 species were identified: *Aphanomyces cladogamus, Globisporangium acanthophoron, G. irregulare, G. ultimum, G. ultimum* var*. sporangiiferum, Phytophthora sojae, Phytopythium cucurbitacearum, Pythium angustatum, P. aphanidermatum, P. deliense, P. myriotylum*, and *P. oopapillum* (Figure 6). However, the species *Phytopythium* sp. "*amazonianum*" and *Pythium vanterpoolii* were not virulent towards soybean, with mean DSI of 11.25% and 10.83% respectively. Thus, the last two species were considered as non-pathogenic to soybeans.

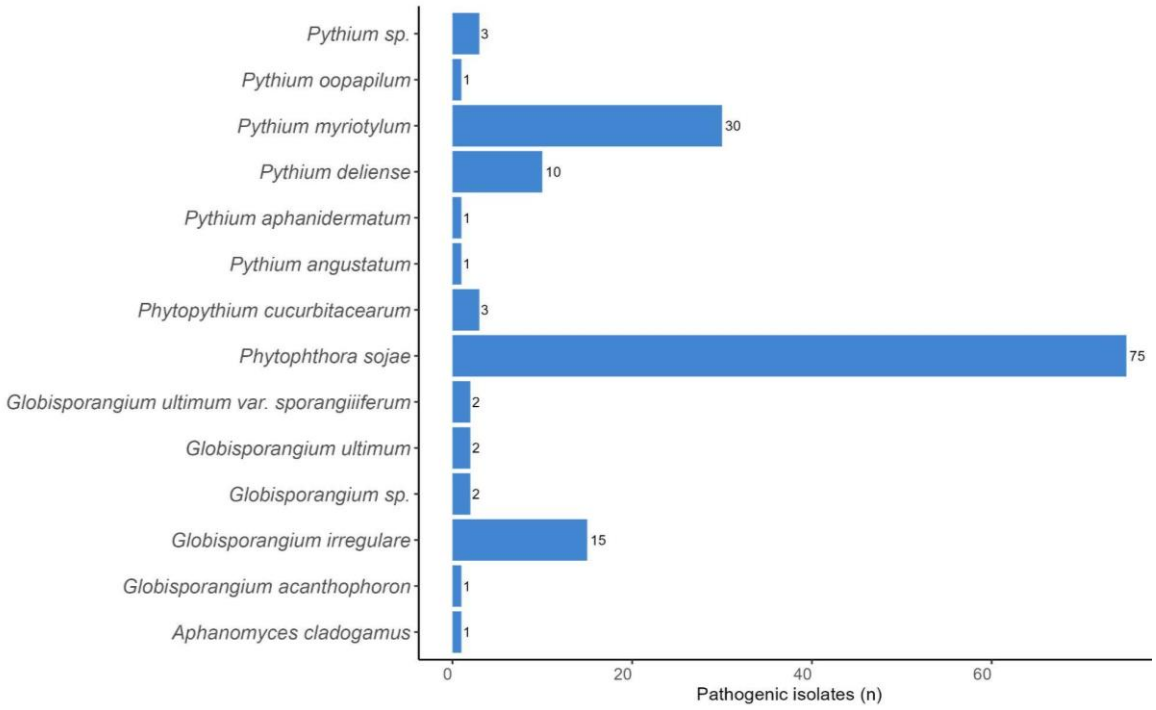

Figure 6. Distribution of the number of pathogenic isolates affecting soybean among various species of oomycetes.The bar chart displays the count of pathogenic isolates (n) for each observed species.

3.4. Sensitivity to fungicides

Oxathiapiprolin, zoxamide, and cyazofamid had no inhibitory effect on growth of *Pythium* and *Globisporangium* isolates. Consequently, the calculation of EC_{50} values for these active ingredients was not possible. However, when mefenoxam was assessed, there was marked inhibition of mycelial growth of all oomycetes isolates (Table 2). Using the W2.4() model with a "delta" range, we estimated the EC_{50} values for mefenoxam.

Table 2. Effective concentration of mefenoxam (Metalaxyl-M) that reduced mycelial growth by 50% for different species of oomycetes tested *in vitro*.

Specie	Estimate EC_{50} (µg/ml)	[1]SE (µg/ml)	[2]CI_{Lower}	[3]CI_{Upper}
Globisporangium irregulare	1.01	0.06	0.88	1.13
Globisporangium ultimum	0.81	0.29	0.21	1.40
Pythium aphanidermatum	31.64	7.30	16.42	46.80
Pythium deliense	0.70	0.05	0.59	0.80
Pythium myriotylum	81.67	11.74	57.28	106.05

[1]SE: Standard error; [2]CI_{Lower}: Lower limit of the 95% confidence interval; [3]CI_{Upper}: Upper limit of the 95% confidence interval.

4. Discussion

Pythium-like and *Phytophthora sojae* can seriously compromise soybean yields worldwide due to the damage these pathogens cause during the initial stages of the crop development. *Pythium*-like are "sneaky" soybean stand reduction pathogens that can pass unnoticed up to the moment that severe plant emergence failures are visible in the field. On the other hand, *P. sojae* usually gets the "spotlight" because it often causes root and stem rot in plants already established in the field. These two groups of oomycetes, one generalist, the *Pythium*-like, and the specialist *P. sojae* must be considered in management programs to avoid crop losses.

The findings of this study offer a comprehensive view into the diverse range of oomycetes present in the Center-South region of Brazil that are a highly underestimated threat for soybean crops. The pathogenicity tests were strategically adapted based on the group of pathogens; i.e. *Phytophthora sojae* and *Pythium*-like isolates. This decision was based on the understanding that *Phytophthora sojae* is the primary causal agent for root and stem rot in soybean and this plant species is its primary host. Thus, the pathogenicity assay for *P. sojae* isolates played a more straightforward role, essentially serving to fulfill requirements. In contrast, for the

remaining *Pythiaceous* isolates, the pathogenicity assay took on a more comprehensive and detailed approach. Both the Petri dish assay and the seedling pathogenicity assay yielded consistent results (Supplementary Figure 2B and 4), and due to the simplicity of the Petri dish assay and effectiveness for assessing the pathogenic potential of various *Pythiaceous* species (Broders et al. 2007), this method was chosen to continue the evaluation of isolates (Supplementary Figure 5).

Twelve pathogenic oomycete species were identified through the analysis of ITS and COI metabarcodes, as well as PCR-based diagnostics for *P. sojae* isolates. The majority of isolates belong to the genera *Phytophthora*, *Pythium*, and *Globisporangium*. Together, these isolates accounted for 91.37% of the oomycetes pathogenic to soybeans recovered in the present work. This finding is consistent with those reported in previous studies that employed a metagenomic approach to investigate the ecology and diversity of oomycetes in cultivated areas (Rojas et al. 2017; Noel et al. 2020; Navarro et al. 2021; Gahagan et al. 2023) and in those that employed baiting and culture-based approaches (Broders et al. 2007; Radmer et al. 2017; Broders et al. 2009).

Late reviews of oomycete taxonomy have led to the reclassification of pathogens previously considered as *Pythium sensu lato* (s.l.). This taxonomic adjustment was based on phylogenetic analyses and involved the division of the genus into distinct categories, including *Pythium sensu stricto* (s.s.), *Elongisporangium*, *Globisporangium*, *Phytopythium* (*Ovatisporangium*), and *Pilasporangium* (Uzuhashi et al. 2010; Nguyen et al. 2022). Thus, species such as the former *Py. irregulare* and *Py. ultimum*, are now recognized as *G. irregulare* and *G. ultimum*, respectively (Uzuhashi et al. 2010; Nguyen et al. 2022; Gahagan et al. 2023). As a practical consequence, current official lists of *Pythium*-like species associated with soybean in a country or in

a region must be updated. Additionally, quarantine procedures and even regulatory issues related to agrichemicals registered to be used to control seed rot must be revised.

There was remarkable variation in virulence among species of the *Pythium* (s.s.) group. *Pythium myriotylum* stood out as the most virulent, displaying the highest severity index (mean DSI: 90.24), followed by *Py. oopapilum* (mean DSI: 73.75) and *Py. aphanidermatum* (mean DSI: 70.50), which also caused severe rot in soybean seeds. *Py. deliense* was pathogenic to soybeans but less virulent than the other species tested (mean DSI: 39.45). *P. vanterpoolii*, did not cause disease in soybeans.

Similar observations regarding the virulence of *P. myriotylum* on soybeans have been reported in studies conducted in Japan and China (Tomioka et al. 2013; Feng et al. 2020). In these studies, not only was *P. myriotylum* one of the most prevalent species, but also the most virulent (Tomioka et al. 2013; Feng et al. 2020). However, there are few studies addressing the virulence of this particular species in Western countries. In Brazil, for instance, although *P. myriotylum* has been previously reported as causing diseases in weeds found in commercial vegetable gardens (Barboza et al. 2022), there is a lack of information regarding its specific impact on soybean crops. Recently, a study identified various species of *Globisporangium* and *Pythium* as responsible for causing damping-off in soybeans, but *P. myriotylum* was not listed (Molin et al. 2021). The detection of *P. myriotylum* in the current study and its absence in previous research may be attributed to the ecological adaptation of this pathogen to potential environmental changes or even to some yet unknown ecological requirements. This existing gap in the understanding of the ecological requirements of *P. myriotylum* hinders the precise correlation between environmental changes and its current prevalence. Targeted investigations are needed to unravel the environmental

preferences, potential ecological interactions, and factors shaping the distribution of *P. myriotylum* for a comprehensive understanding of its behavior and impact on soybean crops and other agricultural environments.

Among the four fungicides examined, only metalaxyl inhibited growth of the majority of oomycete species evaluated in this study. This finding was similar to the results obtained by Molin et al. (2021), which found that metalaxyl was the most effective compound controlling *Globisporangium* spp. in Brazil. In contrast, oxathiapiprolin, zoxamide, and cyazofamid were not efficacious in suppressing the growth of Pythiaceous isolates. In line with these findings, a study by Miao et al. (2016) reported similar observations regarding the effectiveness of oxathiapiprolin. The effectiveness of oxathiapiprolin against *Py. aphanidermatum* and *Py. deliense* was limited, with less than 50% inhibition ratio at 50 µ/mL (Miao et al. 2016).

Contrary to what has been reported in several studies indicating that zoxamide and cyazofamid can inhibit the growth of various *Pythium* species, no such effect was observed herein (Doherty and Roberts 2022, Lookabaugh et al. 2021). However, it is worth emphasizing that certain oomycetes activate an alternative respiratory pathway (AOX) in response to cyazofamid-induced complex III enzyme inhibition (Mitani et al. 2001). In the experiments conducted in the present study, no salicylhydroxamic acid, an AOX inhibitor, was added to the oomicide-amended medium, which could potentially have influenced the observed results.

A range of oomycetes can pose a threat to soybean seedlings. The presence of seed rot, root rot, and damping-off in soybean plants within the Central-South region of Brazil is associated not only with *Phytophthora* but also with *Globisporangium* and *Pythium*, highlighting the multiple challenges faced in protecting soybean crops. Overall, there is variation in the sensitivity of the oomycetes isolates, suggesting that

a combination of active ingredients must be adopted to reduce infections caused by Oomycetes and protect soybean seeds and seedlings at early stages of crop development.

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The authors declare that they have no conflict of interest.

5. References

Alves, K. dos S. 2020. ec50estimator: An automated way to estimate EC50 for stratified datasets. Available at: https://cran.r-project.org/web/packages/ec50estimator/index.html.

Barboza, E. A., Cabral, C. S., Rossato, M., Martins, F. H. S. R., and Reis, A. 2022. *Pythium* and *Phytopythium* species associated with weeds collected in vegetable production fields in Brazil. Lett Appl Microbiol. 74.

Batista, I. C. A., Silva, M. P. C., Silva Junior, A. L., Arriagada, M. P. G., Camargo, M. P. de, Figueiredo, A., Hora Junior, B.T., Mizubuti, E. S. 2023. A shift in pathotype diversity and complexity of *Phytophthora sojae* in Brazil. Plant Dis. 107:7, 1968-1972.

Bienapfl, J. C., Malvick, D. K., and Percich, J. A. 2011. Specific molecular detection of *Phytophthora sojae* using conventional and real-time PCR. Fungal Biol. 115:733–740.

Broders, K. D., Lipps, P. E., Paul, P. A., and Dorrance, A. E. 2007. Characterization of *Pythium* spp. associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91.

Broders, K. D., Wallhead, M. W., Austin, G. D., Lipps, P. E., Paul, P. A., Mullen, R. W., et al. 2009. Association of soil chemical and physical properties with *Pythium s*pecies diversity, community composition, and disease incidence. Phytopathology. 99:957–967.

Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G., and Brasier, C. M. 2000. A molecular phylogeny of phytophthora and related oomycetes. Fungal Genetics and Biology. 30.

Costamilan, L. M., Bonato, E. R., Urben, A. F., Matsouka, K., and Vanetti, C. A. 1996. Occurrence of *Phytophthora sojae* in Brazil. Fitopatol. Bras. 21:395.

Doherty, J. R., and Roberts, J. A. 2022. Investigating chemical and biological control applications for *Pythium* root rot prevention and impacts on creeping bentgrass putting green rhizosphere bacterial communities. Plant Dis. 106.

Dorrance, A. E., Jia, H., and Abney, T. S. 2004. Evaluation of soybean differentials for their interaction with *Phytophthora sojae*. Plant Heal. Prog. 5:9

Dorrance, A., and Grünwald, N. J. 2008. *Phytophthora sojae*: Diversity among and within populations. oomycete genetics and genomics: diversity, interactions, and research tools. :197–212.

Dorrance, A. E. 2018. Management of *Phytophthora sojae* of soybean: a review and future perspectives. Canadian Journal of Plant Pathology, 40(2), 210–219.

Faostat. 2023. Food and Agriculture Organization of the United Nations. Available at: Available at: http://faostat.fao.org.

Feng, H., Chen, J., Yu, Z., Li, K., Li, Z., Li, Y., et al. 2020. Pathogenicity and fungicide sensitivity of *Pythium* and *Phytopythium* spp. associated with soybean in the Huang-Huai region of China. Plant Pathol. 69.

Gahagan, A. C., Shi, Y., Radford, D., Morrison, M. J., Gregorich, E., Aris-Brosou, S., et al. 2023. Long-term tillage and crop rotation regimes reshape soil-borne oomycete communities in soybean, corn, and wheat production systems. Plants. 12:2338.

Grijalba, P. E., Ridao, A. del C., and Steciow, M. M. 2021. Oomycetes species associated with soybean in Buenos Aires Province (Argentina). Phytoparasitica 49:1027–1042.

Guriqbal, S., and Shivakumar, B. G. 2010. The role of soybean in agriculture. In The soybean: botany, production and uses, Wallingford: CABI, p. 24–47.

Haas, J. H., and Buzzell, R. I. 1976. New races 5 and 6 of *Phytophthora megasperma* var. *sojae* and differential reactions of soy-bean cultivars for races 1 to 6. Phytopathology 66:1361-1362.

Kaster, M and Farias, J. R. B. 2012. Regionalização dos testes de valor de cultivo e uso e da indicação de cultivares de soja: Terceira Aproximação. Londrina: Embrapa Soja - Documentos.

Kirkpatrick, M. T., Rothrock, C. S., Rupe, J. C., and Gbur, E. E. 2006. The effect of *Pythium ultimum* and soil flooding on two soybean cultivars. Plant Dis. 90.

Lookabaugh, E. C., Kerns, J. P., and Shew, B. B. 2021. Evaluating fungicide selections to manage pythium root rot on poinsettia cultivars with varying levels of partial resistance. Plant Dis. 105.

Miao, J., Dong, X., Lin, D., Wang, Q., Liu, P., Chen, F., et al. 2016. Activity of the novel fungicide oxathiapiprolin against plant-pathogenic oomycetes. Pest Manag Sci. 72:1572–1577.

Mitani, S., Araki, S., Takii, Y., Ohshima, T., Matsuo, N., and Miyoshi, H. 2001. The biochemical mode of action of the novel selective fungicide cyazofamid: Specific inhibition of mitochondrial complex III in *Phythium spinosum*. Pestic Biochem Physiol. 71.

Molin, C., Ribeiro, N. R., Matsumoto, M. N., Fernando Giasson, N., Brollo, J., Zanardo, B., et al. 2021. Damping-off of soybean in southern Brazil can be associated with different species of *Globisporangium* spp. and *Pythium* spp. Plant Pathol. 70:1686–1694.

Navarro, K. A., Wijeratne, S., Culman, S., Benitez, M. S., and Dorrance, A. E. 2021. Comparison of the species communities of *Phytophthora*, *Pythium*, and *Phytopythium* associated with soybean genotypes in high disease environments in Ohio. Phytobiomes J. 5.

Nguyen, H. D. T., Dodge, A., Dadej, K., Rintoul, T. L., Ponomareva, E., Martin, F. N., et al. 2022. Whole genome sequencing and phylogenomic analysis show support for the splitting of genus *Pythium*. Mycologia. 114.

Noel, Z. A., Chang, H. X., and Chilvers, M. I. 2020. Variation in soybean rhizosphere oomycete communities from Michigan fields with contrasting disease pressures. Applied Soil Ecology. 150:103435.

Nowicki, M., Foolad, M. R., Nowakowska, M., and Kozik, E. U. 2012. Potato and tomato late blight caused by *Phytophthora infestans*: an overview of pathology and resistance breeding. Plant disease, 96: 4-17.

Radmer, L., Anderson, G., Malvick, D. M., Kurle, J. E., Rendahl, A., and Mallik, A. 2017. *Pythium*, *Phytophthora*, and *Phytopythium* spp. associated with soybean in Minnesota, their relative aggressiveness on soybean and corn, and their sensitivity to seed treatment fungicides. Plant Dis. 101:62–72.

Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. PLoS One 10, e0146021.

Robideau, G. P., De Cock, A. W. A. M., Coffey, M. D., Voglmayr, H., Brouwer, H., Bala, K., et al. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Mol Ecol Resour. 11.

Rojas, J. A., Jacobs, J. L., Napieralski, S., Karaj, B., Bradley, C. A., Chase, T., et al. 2017. Oomycete species associated with soybean seedlings in North America—Part II: diversity and ecology in relation to environmental and edaphic factors. Phytopathology. 107:293–304.

Sapkota, R., and Nicolaisen, M. 2015. An improved high throughput sequencing method for studying oomycete communities. J Microbiol Methods. 110:33–39.

Schena, L., Duncan, J. M., and Cooke, D. E. L. 2008. Development and application of a PCR-based “molecular tool box” for the identification of *Phytophthora* species damaging forests and natural ecosystems. Plant Pathol. 57.

Tomioka, K., Takehara, T., Osaki, H., Sekiguchi, H., Nomiyama, K., and Kageyama, K. 2013. Damping-off of soybean caused by *Pythium myriotylum* in Japan. Journal of General Plant Pathology. 79.

Tyler, B. M., and Gijzen, M. 2014. The *Phytophthora sojae* genome sequence: foundation for a revolution. In Genomics of Plant-Associated Fungi and Oomycetes: Dicot Pathogens, Springer-Verlag Berlin Heidelberg, p. 133–157.

Uzuhashi, S., Tojo, M., and Kakishima, M. 2010. Phylogeny of the genus *Pythium* and description of new genera. Mycoscience. 51.

Zhang, Z., Xu, Y., Song, G., Gao, X., Zhao, Y., Jia, M., Chen, Y., Suo, B., Chen, Q., Wu, D., Wu, W., Wen, J. 2019. *Phytophthora soja*e zoospores differ in chemotaxis to the root and root exudates of host soybean and nonhost common bean. J. Gen. Plant Pathol. 85, 201–210.

Zhang, X., Johnson, C., and Reed, D. 2021. Management of *Pythium myriotylum* in tobacco transplant production greenhouses. Plant Health Progress, 2:250-259.

Supplementary data

CHAPTER 1 - Oomycetes and the devastating effects on soybean crop health

Authors: I. C. A. Batista, A. L. Silva Junior, G. C. F. de Melo, A. F. Silva, E. S. G. Mizubuti

Supplementary Table 1. List of primers used in the PCR reactions.

Sequence ID	Sequence 5'-3'	Target gene	Ref.
YPh1F	CGACCATKGGTGTGGACTTT	*Ypt1*	Schena et al. 2008[1]
YPh2R	ACGTTCTCMCAGGCGTATCT		
OomCoxI-Levup	TCAWCWMGATGGCTTTTTTCAAC	*COI*	Robideau et al. 2011[2]
OomCoxI-Levlo	CYTCHGGRTGWCCRAAAAACCAAA		
PSOJF1	GCCTGCTCTGTGTGGCTGT	*ITS*	Bienapfl et al. 2011[3]
PSOJR1	GGTTTAAAAAGTGGGCTCATGATC		
ITS4	TCCTCCGCTTATTGATATGC	*ITS*	Grünwald et at. 2013[4]
ITS6	GAAGGTGAAGTCGTAACAAGG		

[1]Schena, L., Duncan, J. M., and Cooke, D. E. L. 2008. Development and application of a PCR-based "molecular tool box" for the identification of Phytophthora species damaging forests and natural ecosystems. Plant Pathol. 57.

[2]Robideau, G. P., De Cock, A. W. A. M., Coffey, M. D., Voglmayr, H., Brouwer, H., Bala, K., et al. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Mol Ecol Resour. 11.

[3]Bienapfl, J. C., Malvick, D. K., and Percich, J. A. 2011. Specific molecular detection of Phytophthora sojae using conventional and real-time PCR. Fungal Biol. 115:733–740.

[4]Grünwald, N. J., Martin, F. N., Larsen, M. M., Sullivan, C. M., Press, C. M., Coffey, M. D., et al. 2011. Phytophthora-ID.org: A sequence-based phytophthora identification tool. Plant Dis. 95.

Supplementary Table 2. Geographical information, year, bait method used and species of oomycetes recovered from soybean-producing field soil samples.

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy001	894-2	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	Pathogenic	*Phytopythium cucurbitacearum*
UFV-oomy002	894-2	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	Pathogenic	*Phytopythium cucurbitacearum*
UFV-oomy003	894-2	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	Pathogenic	*Phytopythium cucurbitacearum*
UFV-oomy004	894-2	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	Pathogenic	*Phytopythium cucurbitacearum*
UFV-oomy005	4101-2	Leaf	Williams	2020	Xanxerê	SC	103	-26.7805	-52.4512	Pythium-like	Pathogenic	*Globisporangium* sp.
UFV-oomy006	3173-3	Leaf	Williams	2020	Condor	RS	102	-28.0334	-53.5621	Pythium-like	NT[2]	-
UFV-oomy007	912-1	Leaf	Williams	2020	Campo Mourão	PR	203	-23.9954	-52.3171	Pythium-like	Pathogenic	*Globisporangium* sp.
UFV-oomy008	1002-3	Leaf	Williams	2020	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	Pathogenic	*Phytopythium cucurbitacearum*
UFV-oomy009	4237-3	Leaf	Williams	2020	São Miguel das Missões	RS	102	-28.4319	-54.5818	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy010	911-4	Leaf	Williams	2020	Presidente Castelo Branco	SC	202	-23.3106	-52.2136	Pythium-like	NT	-
UFV-oomy011	4239-3	Leaf	Williams	2020	Catuípe	RS	102	-28.1140	-54.0604	Pythium-like	Pathogenic	-
UFV-oomy012	4103-1	Leaf	Williams	2020	Coxilha	RS	103	-28.0979	-52.2718	Pythium-like	NT	-
UFV-oomy013	4101-3	Leaf	Williams	2020	Xanxerê	SC	103	-26.7805	-52.4512	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy014	1001-5	Leaf	Williams	2020	Aral Moreira	MS	202	-22.8871	-55.6046	Pythium-like	Pathogenic	-
UFV-oomy015	1537-2	Leaf	Williams	2020	Mauá da Serra	PR	201	-23.8623	-51.3270	Pythium-like	NT	-
UFV-oomy016	895-3	Leaf	Williams	2020	Rio Brilhante	MS	204	-21.6613	-54.6299	Pythium-like	NT	-
UFV-oomy017	3661-2	Leaf	Williams	2020	Não-Me-Toque	RS	102	-28.4579	-52.822	Pythium-like	NT	-
UFV-oomy018	1537-1	Leaf	Williams	2020	Mauá da Serra	PR	203	-23.8623	-51.3270	Pythium-like	NT	-
UFV-oomy019	895-4	Leaf	Williams	2020	Rio Brilhante	MS	204	-21.6613	-54.6299	Pythium-like	NT	-
UFV-oomy020	894-5	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	NT	-
UFV-oomy021	894-3	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	NT	-
UFV-oomy022	4102-2	Leaf	Williams	2020	Chapecó	SC	103	-27.0199	-52.7248	Pythium-like	NT	-
UFV-oomy023	4103-3	Leaf	Williams	2020	Coxilha	RS	103	-28.0979	-52.2718	Pythium-like	NT	-
UFV-oomy024	2682-3	Leaf	Williams	2020	Ponta Grossa	PR	104	-25.2488	-50.0867	Pythium-like	NT	-
UFV-oomy025	4104-4	Leaf	Williams	2020	Quatro Irmãos	RS	103	-27.9125	-52.5259	Pythium-like	NT	-
UFV-oomy026	13-1	Leaf	Williams	2020	Terra Roxa	PR	201	-24.2610	-54.1058	Pythium-like	Non-pathogenic	-
UFV-oomy027	895-5	Leaf	Williams	2020	Rio Brilhante	MS	204	-21.6613	-54.6299	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy028	894-7	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	NT	-
UFV-oomy029	1537-4	Leaf	Williams	2020	Mauá da Serra	PR	203	-23.8623	-51.3270	Pythium-like	NT	-
UFV-oomy030	2682-4	Leaf	Williams	2020	Ponta Grossa	PR	104	-25.2488	-50.0867	Pythium-like	NT	-
UFV-oomy031	3661-1	Leaf	Williams	2020	Não-Me-Toque	RS	102	-28.4579	-52.822	Pythium-like	NT	-
UFV-oomy032	4103-3	Leaf	Williams	2020	Coxilha	RS	103	-28.0979	-52.2718	Pythium-like	NT	-
UFV-oomy033	4104-3	Leaf	Williams	2020	Quatro Irmãos	RS	103	-27.9125	-52.5259	Pythium-like	NT	-
UFV-oomy034	1002-2	Leaf	Williams	2020	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy035	15-1	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy036	15-3	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy037	1001-3	Leaf	Williams	2020	Aral Moreira	MS	202	-22.8871	-55.6046	Pythium-like	NT	-
UFV-oomy038	4105-4	Leaf	Williams	2020	Ibiaçá	RS	103	-28.0916	-51.8753	Pythium-like	NT	-
UFV-oomy039	1001-1	Leaf	Williams	2020	Aral Moreira	MS	202	-22.8871	-55.6046	Pythium-like	NT	-
UFV-oomy040	2680-5	Leaf	Williams	2020	Itararé	SP	104	-24.0740	-49.3089	Pythium-like	NT	-
UFV-oomy041	3661-3	Leaf	Williams	2020	Não-Me-Toque	RS	102	-28.4579	-52.822	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy042	4103-4	Leaf	Williams	2020	Coxilha	RS	103	-28.0979	-52.2718	Pythium-like	NT	-
UFV-oomy043	4099-3	Leaf	Williams	2020	Abelardo Luz	SC	103	-26.5662	-52.2771	Pythium-like	NT	-
UFV-oomy044	4104-2	Leaf	Williams	2020	Quatro Irmãos	RS	103	-27.9125	-52.5259	Pythium-like	NT	-
UFV-oomy045	1002-3	Leaf	Williams	2020	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy046	2680-4	Leaf	Williams	2020	Itararé	SP	104	-24.0740	-49.3089	Pythium-like	NT	-
UFV-oomy047	2682-2	Leaf	Williams	2020	Ponta Grossa	PR	104	-25.2488	-50.0867	Pythium-like	NT	-
UFV-oomy048	4237-1	Leaf	Williams	2020	São Miguel das Missões	RS	102	-28.4319	-54.5818	Pythium-like	NT	-
UFV-oomy049	4237-2	Leaf	Williams	2020	São Miguel das Missões	RS	102	-28.4319	-54.5818	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy050	4099-1	Leaf	Williams	2020	Abelardo Luz	SC	103	-26.5662	-52.2771	Pythium-like	NT	-
UFV-oomy051	911-3	Leaf	Williams	2020	Presidente Castelo Branco	SC	202	-23.3106	-52.2136	Pythium-like	NT	-
UFV-oomy052	4105-1	Leaf	Williams	2020	Ibiaçá	RS	103	-28.0916	-51.8753	Pythium-like	NT	-
UFV-oomy053	2682-1	Leaf	Williams	2020	Ponta Grossa	PR	104	-25.2488	-50.0867	Pythium-like	NT	-
UFV-oomy054	911-2	Leaf	Williams	2020	Presidente Castelo Branco	SC	202	-23.3106	-52.2136	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy055	894-6	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	NT	-
UFV-oomy056	895-6	Leaf	Williams	2020	Rio Brilhante	MS	204	-21.6613	-54.6299	Pythium-like	NT	-
UFV-oomy057	1001-2	Leaf	Williams	2020	Aral Moreira	MS	202	-22.8871	-55.6046	Pythium-like	NT	-
UFV-oomy058	13-4	Leaf	Williams	2020	Terra Roxa	PR	201	-24.2610	-54.1058	Pythium-like	NT	-
UFV-oomy059	13-3	Leaf	Williams	2020	Terra Roxa	PR	201	-24.2610	-54.1058	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy060	1002-4	Leaf	Williams	2020	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy061	13-2	Leaf	Williams	2020	Terra Roxa	PR	201	-24.2610	-54.1058	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy062	11327-1	Leaf	Williams	2020	Jurandá	PR	201	-24.4167	-52.8482	Pythium-like	NT	-
UFV-oomy063	896-3	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy064	896-8	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy065	11327-6	Leaf	Williams	2020	Jurandá	PR	201	-24.4167	-52.8482	Pythium-like	NT	-
UFV-oomy066	893-5	Leaf	Williams	2020	Dourados	MS	204	-22.2218	-54.8064	Pythium-like	NT	-
UFV-oomy067	896-2	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy068	892-1	Leaf	Williams	2020	Sidrolândia	MS	204	-21.2412	-55.5519	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy069	896-1	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy070	11327-2	Leaf	Williams	2020	Jurandá	PR	201	-24.4167	-52.8482	Pythium-like	NT	-
UFV-oomy071	11327-3	Leaf	Williams	2020	Jurandá	PR	201	-24.4167	-52.8482	Pythium-like	NT	-
UFV-oomy072	896-1	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy073	3173-1	Leaf	Williams	2020	Condor	RS	103	-28.0334	-53.5621	Pythium-like	Non-pathogenic	-
UFV-oomy074	3173-3	Leaf	Williams	2020	Condor	RS	103	-28.0334	-53.5621	Pythium-like	NT	-
UFV-oomy075	15-3	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy076	1001-5	Leaf	Williams	2020	Aral Moreira	MS	202	-22.8871	-55.6046	Pythium-like	NT	-
UFV-oomy077	4099-4	Leaf	Williams	2020	Abelardo Luz	SC	103	-26.5662	-52.2771	Pythium-like	NT	-
UFV-oomy078	915-1	Leaf	Williams	2020	Bela Vista do Paraiso	PR	203	-22.9537	-51.3657	Pythium-like	Pathogenic	-
UFV-oomy079	1537-3	Leaf	Williams	2020	Mauá da Serra	PR	203	-23.8623	-51.3270	Pythium-like	NT	-
UFV-oomy080	895-2	Leaf	Williams	2020	Rio Brilhante	MS	204	-21.6613	-54.6299	Pythium-like	NT	-
UFV-oomy081	895-4	Leaf	Williams	2020	Rio Brilhante	MS	204	-21.6613	-54.6299	Pythium-like	NT	-
UFV-oomy082	4103-1	Leaf	Williams	2020	Coxilha	RS	103	-28.0979	-52.2718	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy083	3661-1	Leaf	Williams	2020	Não-Me-Toque	RS	102	-28.4579	-52.822	Pythium-like	NT	-
UFV-oomy084	4102-1	Leaf	Williams	2020	Chapecó	SC	103	-27.0199	-52.7248	Pythium-like	NT	-
UFV-oomy085	4099-2	Leaf	Williams	2020	Abelardo Luz	SC	103	-26.5662	-52.2771	Pythium-like	NT	-
UFV-oomy086	911-4	Leaf	Williams	2020	Presidente Castelo Branco	SC	202	-23.2849	-52.1658	Pythium-like	NT	-
UFV-oomy087	4105-3	Leaf	Williams	2020	Ibiaçá	RS	103	-28.0916	-51.8753	Pythium-like	NT	-
UFV-oomy088	4105-2	Leaf	Williams	2020	Ibiaçá	RS	103	-28.0916	-51.8753	Pythium-like	NT	-
UFV-oomy089	894-3	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	NT	-
UFV-oomy090	3661-4	Leaf	Williams	2020	Não-Me-Toque	RS	102	-28.4579	-52.822	Pythium-like	NT	-
UFV-oomy091	895-2	Leaf	Williams	2020	Rio Brilhante	MS	204	-21.6613	-54.6299	Pythium-like	NT	-
UFV-oomy092	3173-1	Leaf	Williams	2020	Condor	RS	103	-28.0334	-53.5621	Pythium-like	NT	-
UFV-oomy093	4104-1	Leaf	Williams	2020	Quatro Irmãos	RS	103	-27.9125	-52.5259	Pythium-like	NT	-
UFV-oomy094	896-1	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy095	896-3	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy096	896-1	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy097	896-1	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy098	896-2	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy099	913-1	Leaf	Williams	2020	Andira	PR	203	-23.0026	-50.3603	Pythium-like	NT	-
UFV-oomy100	894-1	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	NT	-
UFV-oomy101	894-8	Leaf	Williams	2020	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	NT	-
UFV-oomy102	11328-3	Leaf	Williams	2020	Sao Miguel do Iguacu	PR	201	-25.4431	-54.3079	Pythium-like	NT	-
UFV-oomy103	11328-3	Leaf	Williams	2020	Sao Miguel do Iguacu	PR	201	-25.4431	-54.3079	Pythium-like	NT	-
UFV-oomy104	RS01	Leaf	Williams	2020	Santa Maria	RS	101	-29.6914	-53.8008	Pythium-like	NT	-
UFV-oomy105	15-2	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy106	15-1	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy107	15-4	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy108	15-3	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy109	15-2	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy110	15-1	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy111	15-3	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy112	15-4	Leaf	Williams	2020	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy113	4102-3	Leaf	Williams	2020	Chapecó	SC	103	-27.0199	-52.7248	Pythium-like	NT	-
UFV-oomy114	4103-1	Leaf	Williams	2020	Coxilha	RS	103	-28.0979	-52.2718	Pythium-like	NT	-
UFV-oomy115	892-6	Leaf	Williams	2020	Sidrolândia	MS	204	-21.2412	-55.5519	Pythium-like	NT	-
UFV-oomy116	3173-4	Leaf	Williams	2020	Condor	RS	103	-28.0334	-53.5621	Pythium-like	NT	-
UFV-oomy117	3661-1	Leaf	Williams	2020	Não-Me-Toque	RS	102	-28.4579	-52.822	Pythium-like	NT	-
UFV-oomy118	911-4	Leaf	Williams	2020	Presidente Castelo Branco	SC	202	-23.3106	-52.2136	Pythium-like	NT	-
UFV-oomy119	4099-2	Leaf	Williams	2020	Abelardo Luz	SC	103	-26.5662	-52.2771	Pythium-like	NT	-
UFV-oomy120	4099-4	Leaf	Williams	2020	Abelardo Luz	SC	103	-26.5662	-52.2771	Pythium-like	NT	-
UFV-oomy121	915-1	Leaf	Williams	2020	Bela Vista do Paraiso	PR	203	-22.9537	-51.3657	Pythium-like	NT	-
UFV-oomy122	1537-3	Leaf	Williams	2020	Mauá da Serra	PR	203	-23.8623	-51.3270	Pythium-like	NT	-
UFV-oomy123	4239-1	Leaf	Williams	2020	Catuípe	RS	102	-28.1140	-54.0604	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy124	4239-1	Leaf	Williams	2020	Catuípe	RS	102	-28.1140	-54.0604	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy125	896-3	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy126	896-4	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy127	896-2	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy128	896-1	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy129	896-1	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy130	896-4	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy131	896-3	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy132	896-3	Leaf	Williams	2020	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy133	4	Leaf	Williams	2021	Dourados	MS	204	-22.2218	-54.8064	Pythium-like	NT	-
UFV-oomy134	5	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy135	6-2	Leaf	Williams	2021	Itahum	MS	204	-22.2000	-55.3333	Pythium-like	NT	-
UFV-oomy136	6-1	Leaf	Williams	2021	Itahum	MS	204	-22.2000	-55.3333	Pythium-like	NT	-
UFV-oomy137	6-3	Leaf	Williams	2021	Itahum	MS	204	-22.2000	-55.3333	Pythium-like	NT	-
UFV-oomy138	7	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy139	8	Leaf	Williams	2021	Itaporã	MS	204	-22.0821	-54.7889	Pythium-like	NT	-
UFV-oomy140	11-2	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy141	11-1	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy142	15	Leaf	Williams	2021	Dourados	MS	204	-22.2218	-54.8064	Pythium-like	NT	-
UFV-oomy143	18	Leaf	Williams	2021	Capão do Leão	RS	101	-31.7675	-52.4487	Pythium-like	Pathogenic	-
UFV-oomy144	19-2	Leaf	Williams	2021	Capão do Leão	RS	101	-31.7675	-52.4487	Pythium-like	NT	-
UFV-oomy145	19-1	Leaf	Williams	2021	Capão do Leão	RS	101	-31.7675	-52.4487	Pythium-like	Pathogenic	*Aphanomyces cladogamus*
UFV-oomy146	20	Leaf	Williams	2021	Tapes	RS	101	-30.6742	-51.3966	Pythium-like	Pathogenic	*Pythium* sp.
UFV-oomy147	21-1	Leaf	Williams	2021	Tapes	RS	101	-30.6742	-51.3966	Pythium-like	Pathogenic	*Pythium angustatum*
UFV-oomy148	22-1	Leaf	Williams	2021	Restinga Sêca	RS	101	-28.2203	-54.3389	Pythium-like	NT	–
UFV-oomy149	22-2	Leaf	Williams	2021	Restinga Sêca	RS	101	-28.2203	-54.3389	Pythium-like	NT	-
UFV-oomy150	23	Leaf	Williams	2021	São Sépé	RS	101	-30.1706	-53.5802	Pythium-like	NT	-
UFV-oomy151	24-1	Leaf	Williams	2021	-	-	-	-	-	Pythium-like	NT	-
UFV-oomy152	24-2	Leaf	Williams	2021	-	-	-	-	-	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy153	25	Leaf	Williams	2021	-	-	-	-	-	Pythium-like	NT	-
UFV-oomy154	26-5	Leaf	Williams	2021	-	-	-	-	-	Pythium-like	NT	-
UFV-oomy155	26-3	Leaf	Williams	2021	-	-	-	-	-	Pythium-like	NT	-
UFV-oomy156	26-4	Leaf	Williams	2021	-	-	-	-	-	Pythium-like	NT	-
UFV-oomy157	30-4	Leaf	Williams	2021	Santo Antonio da Platina	PR	201	-23.2982	-50.0694	Pythium-like	NT	-
UFV-oomy158	30-2	Leaf	Williams	2021	Santo Antonio da Platina	PR	201	-23.2982	-50.0694	Pythium-like	NT	-
UFV-oomy159	30-3	Leaf	Williams	2021	Santo Antonio da Platina	PR	201	-23.2982	-50.0694	Pythium-like	NT	-
UFV-oomy160	31-1	Leaf	Williams	2021	Bandeirantes	PR	201	-23.105	-50.3603	Pythium-like	NT	-
UFV-oomy161	33	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy162	34	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy163	35	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy164	36-5	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy165	36-1	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy166	36-4	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy167	026-1	Leaf	Williams	2021						Pythium-like	NT	-
UFV-oomy168	92	Leaf	Williams	2021	Restinga	RS	101	-28.2203	-54.3389	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy169	143-1	Leaf	Williams	2021	Santo Antonio da Platina	PR	201	-23.2982	-50.0694	Pythium-like	NT	-
UFV-oomy170	185-1	Leaf	Williams	2021	Tapes	RS	101	-30.6742	-51.3966	Pythium-like	NT	-
UFV-oomy171	187-1	Leaf	Williams	2021	Tapes	RS	101	-30.6742	-51.3966	Pythium-like	NT	-
UFV-oomy172	189-1	Leaf	Williams	2021	Tapes	RS	101	-30.6742	-51.3966	Pythium-like	NT	-
UFV-oomy173	189-2	Leaf	Williams	2021	Tapes	RS	101	-30.6742	-51.3966	Pythium-like	NT	-
UFV-oomy174	201-1	Leaf	Williams	2021	Caçapava do Sul	RS	101	-30.5164	-53.4868	Pythium-like	NT	-
UFV-oomy175	201-2	Leaf	Williams	2021	Caçapava do Sul	RS	101	-30.5164	-53.4868	Pythium-like	NT	-
UFV-oomy176	287-1	Leaf	Williams	2021	Não-Me-Toque	RS	102	-28.4579	-52.822	Pythium-like	NT	-
UFV-oomy177	287-2	Leaf	Williams	2021	Não-Me-Toque	RS	102	-28.4579	-52.822	Pythium-like	NT	-
UFV-oomy178	287-4	Leaf	Williams	2021	Não-Me-Toque	RS	102	-28.4579	-52.822	Pythium-like	NT	-
UFV-oomy179	1	Leaf	Williams	2021	Caarapó	MS	202	-22.6298	-54.8253	Pythium-like	NT	-
UFV-oomy180	2	Leaf	Williams	2021	Aral Moreira	MS	202	-22.8871	-55.6046	Pythium-like	Pathogenic	*Globisporangium irregulare*

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy181	12	Leaf	Williams	2021	Itahum	MS	204	-22.2000	-55.3333	Pythium-like	NT	-
UFV-oomy182	13	Leaf	Williams	2021	Aral Moreira	MS	202	-22.8871	-55.6046	Pythium-like	NT	-
UFV-oomy183	14	Leaf	Williams	2021	Aral Moreira	MS	202	-22.8871	-55.6046	Pythium-like	NT	-
UFV-oomy184	16	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	Non-pathogenic	-
UFV-oomy185	25-5	Leaf	Williams	2021						Pythium-like	NT	-
UFV-oomy186	27	Leaf	Williams	2021	Bandeirantes	PR	201	-23.105	-50.3603	Pythium-like	NT	-
UFV-oomy187	28	Leaf	Williams	2021	Bandeirantes	PR	201	-23.105	-50.3603	Pythium-like	Pathogenic	*Globisporangium ultimum*
UFV-oomy188	29	Leaf	Williams	2021	Florinia	SP	201	-20.9033	-50.7378	Pythium-like	NT	-
UFV-oomy189	30-5	Leaf	Williams	2021	Santo Antonio da Platina	PR	201	-23.2982	-50.0694	Pythium-like	NT	-
UFV-oomy190	42	Leaf	Williams	2021	Capão do Leão	RS	101	-31.7675	-52.4487	Pythium-like	Pathogenic	*Pythium* sp.
UFV-oomy191	47	Leaf	Williams	2021	Rosário do Sul	RS	101	-30.2434	-54.9218	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy192	52-1	Leaf	Williams	2021	São Vicente do Sul	RS	101	-29.6921	-54.6797	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy193	52-2	Leaf	Williams	2021	São Vicente do Sul	RS	101	-29.6921	-54.6797	Pythium-like	NT	-
UFV-oomy194	53	Leaf	Williams	2021	Dom Pedrito	RS	101	-30.9818	-54.6775	Pythium-like	Non-pathogenic	*Pythium* sp.

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy195	58	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	Pathogenic	*Pythium* sp.
UFV-oomy196	59-5	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy197	59-1	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	Non-pathogenic	*Pythium vanterpoolii*
UFV-oomy198	59-2	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	Non-pathogenic	*Pythium vanterpoolii*
UFV-oomy199	60	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy200	61	Leaf	Williams	2021	Ponta Porã	MS	204	-22.5853	-55.5023	Pythium-like	NT	-
UFV-oomy201	63	Leaf	Williams	2021	Colorado	RS	102	-28.5234	-52.9877	Pythium-like	NT	-
UFV-oomy202	64	Leaf	Williams	2021	Colorado	RS	102	-28.5234	-52.9877	Pythium-like	NT	-
UFV-oomy203	65-5	Leaf	Williams	2021	Cambé	PR	201	-23.2751	-51.2815	Pythium-like	NT	-
UFV-oomy204	65-1	Leaf	Williams	2021	Cambé	PR	201	-23.2751	-51.2815	Pythium-like	NT	-
UFV-oomy205	65-2	Leaf	Williams	2021	Cambé	PR	201	-23.2751	-51.2815	Pythium-like	NT	-
UFV-oomy206	68-1	Leaf	Williams	2021	Maracaju	MS	204	-21.6189	-55.1673	Pythium-like	NT	-
UFV-oomy207	68-2	Leaf	Williams	2021	Maracaju	MS	204	-21.6189	-55.1673	Pythium-like	NT	-
UFV-oomy208	69	Leaf	Williams	2021	Sidrolândia	MS	204	-20.9316	-54.9695	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy209	70	Leaf	Williams	2021	Rio Brilhante	MS	204	-21.8023	-54.5436	Pythium-like	NT	-
UFV-oomy210	71	Leaf	Williams	2021	Sidrolândia	MS	204	-20.9316	-54.9695	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy211	79	Leaf	Williams	2021	Rio Brilhante	MS	204	-21.8023	-54.5436	Pythium-like	NT	-
UFV-oomy212	43	Leaf	Williams	2021	Dilermando de Aguiar	RS	101	-29.7064	-54.2089	Pythium-like	NT	-
UFV-oomy213	72-1	Leaf	Williams	2021	Rio Brilhante	MS	204	-21.8023	-54.5436	Pythium-like	NT	-
UFV-oomy214	72-2	Leaf	Williams	2021	Rio Brilhante	MS	204	-21.8023	-54.5436	Pythium-like	NT	-
UFV-oomy215	72-3	Leaf	Williams	2021	Rio Brilhante	MS	204	-21.8023	-54.5436	Pythium-like	NT	-
UFV-oomy216	79	Leaf	Williams	2021	Rio Brilhante	MS	204	-21.8023	-54.5436	Pythium-like	NT	-
UFV-oomy217	112	Leaf	Williams	2021	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy218	113	Leaf	Williams	2021	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	NT	-
UFV-oomy219	188	Leaf	Williams	2021	Tapes	RS	101	-30.6742	-51.3966	Pythium-like	Pathogenic	*Pythium oopapilum*
UFV-oomy220	189	Leaf	Williams	2021	Tapes	RS	101	-30.6742	-51.3966	Pythium-like	NT	-
UFV-oomy221	226	bioensaio	Williams	2022	Cachoeira do Sul	RS	101	-30.0125	-52.9198	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy222	342	bioensaio	Williams	2022	Cruz Alta	RS	102	-28.6394	-53.6062	Pythium-like	Pathogenic	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy223	368	bioensaio	Williams	2022	Santo Angelo	RS	102	-28.3003	-54.2635	Pythium-like	Pathogenic	*Pythium* sp.
UFV-oomy224	325	bioensaio	Williams	2022	Cruz Alta	RS	102	-28.6394	-53.6062	Pythium-like	Pathogenic	*Pythium aphanidermatum*
UFV-oomy225	334	bioensaio	Williams	2022	Cruz Alta	RS	102	-28.6394	-53.6062	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy226	336	bioensaio	Williams	2022	Dois irmaos das missoes	RS	102	-27.6545	-53.5298	Pythium-like	NT	-
UFV-oomy227	289-1	bioensaio	Williams	2022	Tibagi	PR	103	-24.5018	-50.4416	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy228	298-3	bioensaio	Williams	2022	Tibagi	PR	103	-24.5018	-50.4416	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy229	298-4	bioensaio	Williams	2022	Tibagi	PR	103	-24.5018	-50.4416	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy230	196	bioensaio	Williams	2022	Restinga Sêca	RS	101	-28.2203	-54.3389	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy231	694-2	bioensaio	Williams	2022						Pythium-like	Pathogenic	-
UFV-oomy232	78	bioensaio	Williams	2022	Maracaju	MS	204	-21.6189	-55.1673	Pythium-like	Pathogenic	*Pythium deliense*
UFV-oomy233	102	bioensaio	Williams	2022	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy234	129	bioensaio	Williams	2022	Santa Cecília	SC	103	-26.9608	-50.4154	Pythium-like	Pathogenic	*Pythium deliense*
UFV-oomy235	A15.04.F123	bioensaio	Williams	2022	Cachoeira Dourada	MG	302	-18.4768	-49.4931	Pythium-like	non-pathogenic	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy236	212	bioensaio	Williams	2022	São Vicente do Sul	RS	101	-29.6921	-54.6797	Pythium-like	NT	-
UFV-oomy237	102	bioensaio	Williams	2022	Quarto Centenário	PR	201	-24.2828	-53.0902	Pythium-like	NT	*Pythium myriotylum*
UFV-oomy238	134	bioensaio	Williams	2022	Maracaí	SP	201	-22.6142	-50.6687	Pythium-like	Pathogenic	*Pythium deliense*
UFV-oomy239	199 P3	bioensaio	Williams	2022	Caçapava do Sul	RS	101	-30.5164	-53.4868	Pythium-like	Pathogenic	*Pythium deliense*
UFV-oomy240	199 P1	bioensaio	Williams	2022	Caçapava do Sul	RS	101	-30.5164	-53.4868	Pythium-like	Pathogenic	*Pythium deliense*
UFV-oomy241	199 P2	bioensaio	Williams	2022	Caçapava do Sul	RS	101	-30.5164	-53.4868	Pythium-like	Pathogenic	*Pythium deliense*
UFV-oomy242	116	bioensaio	Williams	2022	Guarapuava	PR	103	-25.3935	-51.4562	Pythium-like	Pathogenic	*Globisporangium ultimum* var. *sporangiiiferum*
UFV-oomy243	306	bioensaio	Williams	2022	Arapoti	PR	103	-24.1453	-49.8188	Pythium-like	Pathogenic	Globisporangium irregulare
UFV-oomy244	136	bioensaio	Williams	2022	Candido Mota	SP	201	-22.7461	-50.3875	Pythium-like	Pathogenic	*Pythium deliense*
UFV-oomy245	163	bioensaio	Williams	2022	Candido Mota	SP	201	-22.7461	-50.3875	Pythium-like	Pathogenic	*Pythium deliense*
UFV-oomy246	191	bioensaio	Williams	2022	São Martinho	RS	102	-28.1739	-48.975	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy247	222	bioensaio	Williams	2022	Senador Salgado Filho	RS	102	-28.0302	-54.5561	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy248	271	bioensaio	Williams	2022	Guarapuava	PR	103	-25.3935	-51.4562	Pythium-like	Pathogenic	*Pythium myriotylum*

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy249	106	bioensaio	Williams	2022	Terra Roxa	PR	201	-24.2610	-54.1058	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy250	123	bioensaio	Williams	2022	Itaberá	SP	103	-23.856	-49.1358	Pythium-like	NT	-
UFV-oomy251	155	bioensaio	Williams	2022	Cambará	PR	201	-23.0372	-50.0714	Pythium-like	Pathogenic	*Pythium deliense*
UFV-oomy252	400-2	bioensaio	Williams	2023	Ibirarema	SP	201	-22.8178	-50.0742	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy253	388	bioensaio	Williams	2023	Santa Cruz do Rio Pardo	SP	203	-22.9077	-49.6176	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy254	440-1	bioensaio	Williams	2023	Ventania	PR	103	-24.2444	-50.2432	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy255	402-1	bioensaio	Williams	2023	Iguaraçu	PR	202	-23.1933	-51.8424	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy256	384-2	bioensaio	Williams	2023	Medianeira	PR	201	-25.2885	-54.1275	Pythium-like	Pathogenic	-
UFV-oomy257	395-1	bioensaio	Williams	2023	Floresta	PR	201	-23.6102	-52.087	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy258	400-1	bioensaio	Williams	2023	Ibirarema	SP	201	-22.8178	-50.0742	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy259	402-2	bioensaio	Williams	2023	Iguaraçu	PR	202	-23.1933	-51.8424	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy260	386-1	bioensaio	Williams	2023	Toledo	PR	201	-24.6665	-53.7828	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy261	384-1	bioensaio	Williams	2023	Medianeira	PR	201	-25.2885	-54.1275	Pythium-like	Pathogenic	*Pythium myriotylum*

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy262	390-2	bioensaio	Williams	2023	Sao Miguel do Iguacu	PR	201	-25.4431	-54.3079	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy263	399	bioensaio	Williams	2023	Santo Antonio da Platina	PR	201	-23.2982	-50.0694	Pythium-like	Pathogenic	*Pythium deliense*
UFV-oomy264	419-1	bioensaio	Williams	2023	Campo Mourão	PR	203	-23.9954	-52.3171	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy265	380	bioensaio	Williams	2023	Ramilandia	PR	201	-25.1161	-54.0400	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy266	425	bioensaio	Williams	2023	Douradina	MS	204	-22.0368	-54.6117	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy267	395	bioensaio	Williams	2023	Floresta	PR	201	-23.6102	-52.087	Pythium-like	Pathogenic	*Pythium* sp.
UFV-oomy268	390-1	bioensaio	Williams	2023	Sao Miguel do Iguacu	PR	201	-25.4431	-54.3079	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy269	172-2	Leaf	Williams	2023	Bela Vista	MS	204	-22.0825	-56.5259	Pythium-like	Pathogenic	*Globisporangium* sp.
UFV-oomy270	487	Leaf	Williams	2023	Uberlandia	MG	303	-18.9113	-48.2622	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy271	381	Leaf	Williams	2023	Santa Helena	PR	201	-24.8641	-54.3302	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy272	417	Leaf	Williams	2023	Boa Esperança	PR	102	-24.2279	-52.7935	Pythium-like	NT	-
UFV-oomy273	172-1	Leaf	Williams	2023	Bela Vista	MS	204	-220825	-56.5259	Pythium-like	Pathogenic	*Globisporangium ultimum* var. *sporangiiiferum*
UFV-oomy274	412	Leaf	Williams	2023	Sertanopolis	PR	201	-23.0597	-51.0332	Pythium-like	NT	-

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy275	488	Leaf	Williams	2023	Cruz Alta	RS	102	-28.6394	-53.6062	Pythium-like	Non-pathogenic	*Phytopythium* sp. "amazonianum"
UFV-oomy276	396	Leaf	Williams	2023	Palotina	PR	201	-24.3213	-53.8304	Pythium-like	Non-pathogenic	*Phytopythium cucurbitacearum*
UFV-oomy277	413	Leaf	Williams	2023	Bela Vista do Paraiso	PR	201	-22.9948	-51.1858	Pythium-like	Pathogenic	*Phytopythium* sp.
UFV-oomy278	442	Leaf	Williams	2023	Pejuçara	RS	102	-28.4222	-53.6557	Pythium-like	Pathogenic	*Globisporangium ultimum*
UFV-oomy279	382	Leaf	Williams	2023	Corbelia	PR	201	-24.798	-53.2927	Pythium-like	Pathogenic	*Globisporangium irregulare*
UFV-oomy280	394	Leaf	Williams	2023	Moreira Sales	PR	201	-24.0443	-53.0066	Pythium-like	Non-pathogenic	*Phytopythium cucurbitacearum*
UFV-oomy281	446	Leaf	Williams	2023	Dourados	MS	204	-22.2218	-54.8064	Pythium-like	NT	*Phytopythium cucurbitacearum*
UFV-oomy282	391-1	Leaf	Williams	2023	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy283	391-2	Leaf	Williams	2023	Quarto Centenário	PR	203	-24.3013	-53.1677	Pythium-like	NT	-
UFV-oomy284	446	Leaf	Williams	2023	Dourados	MS	204	-22.2218	-54.8064	Pythium-like	NT	-
UFV-oomy285	469	Leaf	Williams	2023	Quatro Irmãos	RS	102	-27.8165	-52.4381	Pythium-like	NT	-
UFV-oomy286	392	Leaf	Williams	2023	Jurandá	PR	201	-24.4167	-52.8482	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV-oomy287	460	Leaf	Williams	2023	Maracaju	MS	204	-21.7225	-55.1936	Pythium-like	Pathogenic	*Pythium myriotylum*

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV-oomy288	469	Leaf	Williams	2023	Quatro Irmãos	RS	103	-27.9125	-52.5259	Pythium-like	NT	*Globisporangium* sp.
UFV-oomy289	419	Leaf	Williams	2023	Campo Mourão	PR	203	-23.9954	-52.3171	Pythium-like	Pathogenic	*Globisporangium acanthophoron*
UFV-oomy290	480	Leaf	Williams	2023	Santiago	RS	102	-29.1731	-54.8558	Pythium-like	NT	-
UFV-oomy291	474	Leaf	Williams	2023	Dom Pedrito	RS	101	-30.9818	-54.6775	Pythium-like	NT	-
UFV-oomy292	477	Leaf	Williams	2023	Rosário do Sul	RS	101	-30.2434	-54.9218	Pythium-like	NT	-
UFV-oomy293	478	Leaf	Williams	2023	Cachoeira do Sul	RS	101	-30.0125	-52.9198	Pythium-like	NT	-
UFV-oomy294	486	Leaf	Williams	2023	Capão do Leão	RS	101	-31.7675	-52.4487	Pythium-like	NT	-
UFV-oomy295	487	Leaf	Williams	2023	Uberlandia	MG	303	-18.9113	-48.2622	Pythium-like	Pathogenic	*Pythium myriotylum*
UFV - Ps001	286	Leaf-bait	Williams	2021	Não-Me-Toque	RS	102	-28.4579	-52.8220	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps002	286	Leaf-bait	Williams	2021	Não-Me-Toque	RS	102	-28.4579	-52.8220	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps003	287	Leaf-bait	Williams	2021	Não-Me-Toque	RS	102	-28.4579	-52.8220	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps004	287	Leaf-bait	Williams	2021	Não-Me-Toque	RS	102	-28.4579	-52.8220	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps005	340	Leaf-bait	Williams	2021	Sarandi	RS	102	-27.9548	-52.9152	Phytophthora	Pathogenic	*Phytophthora sojae*

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV - Ps006	286	Leaf-bait	Williams	2021	Não-Me-Toque	RS	102	-28.4579	-52.8220	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps007	188	Leaf-bait	Conquista	2021	Tapes	RS	101	-30.6742	-51.3966	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps008	341	Leaf-bait	Williams	2021	Sarandi	RS	102	-27.9548	-52.9152	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps009	186	Leaf-bait	Conquista	2021	Tapes	RS	101	-30.6742	-51.3966	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps010	341	Leaf-bait	Williams	2021	Sarandi	RS	102	-27.9548	-52.9152	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps011	334	Leaf-bait	Williams	2021	Cruz Alta	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps012	334	Leaf-bait	Williams	2021	Cruz Alta	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps013	322	Leaf-bait	Conquista	2021	Abelardo Luz	SC	102	-26.5672	-52.3339	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps014	322	Leaf-bait	Conquista	2021	Abelardo Luz	SC	102	-26.5672	-52.3339	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps015	325	Leaf-bait	Conquista	2021	Cruz Alta	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps016	325	Leaf-bait	Conquista	2021	Cruz Alta	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps017	325	Leaf-bait	Conquista	2021	Cruz Alta	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps018	318	Leaf-bait	Williams	2021	Cruz Alta	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV - Ps019	318	Leaf-bait	Williams	2021	Cruz Alta	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps020	220-1	Bioassay	Williams - L93-3312	2021	Santo Augusto	RS	102	-27.8515	-53.7772	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps021	220-2	Bioassay	Williams - L93-3312	2021	Santo Augusto	RS	102	-27.8515	-53.7772	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps022	221-2	Bioassay	Williams - L93-3312	2021	Santo Augusto	RS	102	-27.8515	-53.7772	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps023	221-1	Bioassay	Williams - L93-3312	2021	Santo Augusto	RS	102	-27.8515	-53.7772	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps024	340-1	Fruit	-	2021	Sarandi	RS	102	-28.4579	-52.8220	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps025	340-2	Fruit	-	2021	Sarandi	RS	102	-28.4579	-52.8220	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps026	210-1	Bioassay	Williams - L93-3312	2022	Alegrete	RS	101	-29.7848	-55.7757	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps027	210-2	Bioassay	Williams - L93-3312	2022	Alegrete	RS	101	-29.7848	-55.7757	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps028	220-3	Bioassay	Williams - L93-3312	2022	Santo Augusto	RS	102	-27.8515	-53.7772	Phytophthora	Pathogenic	*Phytophthora sojae*

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV - Ps029	220-4	Bioassay	Williams - L93-3312	2022	Santo Augusto	RS	102	-25.3935	-51.4566	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps030	272-2	Bioassay	Williams - L93-3312	2022	Guarapuava	PR	103	-25.3935	-51.4566	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps031	272-1	Bioassay	Williams - L93-3312	2022	Guarapuava	PR	103	-25.3935	-51.4566	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps032	272-3	Bioassay	Williams - L93-3312	2022	Guarapuava	PR	103	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps033	222-3	Bioassay	Williams - L93-3312	2022	Senador Salgado Filho	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps034	340-3	Bioassay	Williams	2022	Sarandi	RS	102	-30.5164	-53.4868	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps035	222-1	Bioassay	Williams - L93-3312	2022	Senador Salgado Filho	RS	102	-30.5164	-53.4868	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps036	199-1	Bioassay	Williams - L93-3312	2022	Caçapava do Sul	RS	101	-30.5164	-53.4868	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps037	200-1	Bioassay	Williams - L93-3312	2022	Caçapava do Sul	RS	101	-30.9818	-54.6775	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps038	200-2	Bioassay	Williams - L93-3312	2022	Caçapava do Sul	RS	101	-30.9818	-54.6775	Phytophthora	Pathogenic	*Phytophthora sojae*

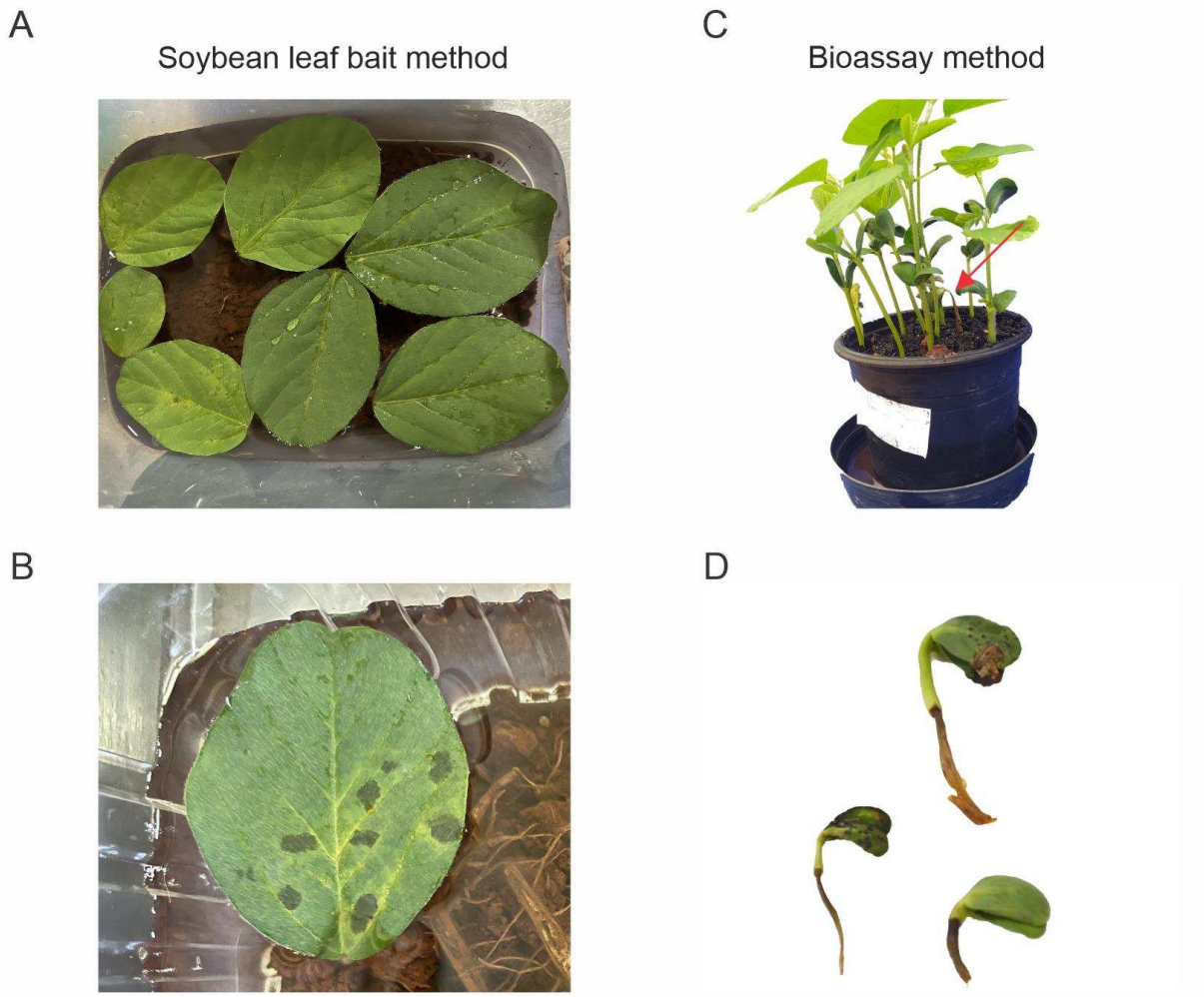
ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV - Ps039	200-3	Bioassay	Williams - L93-3312	2022	Caçapava do Sul	RS	101	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps040	200-4	Bioassay	Williams - L93-3312	2022	Caçapava do Sul	RS	101	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps041	200-5	Bioassay	Williams - L93-3312	2022	Caçapava do Sul	RS	101	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps042	207-1	Bioassay	Williams - L93-3312	2022	Dom Pedrito	RS	101	-30.9818	-54.6775	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps043	207-2	Bioassay	Williams - L93-3312	2022	Dom Pedrito	RS	101	-30.9818	-54.6775	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps044	207-3	Bioassay	Williams - L93-3312	2022	Dom Pedrito	RS	101	-30.9818	-54.6775	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps045	222-3	Bioassay	Williams - L93-3312	2022	Senador Salgado Filho	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps046	222-4	Bioassay	Williams - L93-3312	2022	Senador Salgado Filho	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps047	222-5	Bioassay	Williams - L93-3312	2022	Senador Salgado Filho	RS	102	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps048	202-1	Bioassay	Williams - L93-3312	2022	Santa Margarida do Sul	RS	101	-30.3385	-54.0707	Phytophthora	Pathogenic	*Phytophthora sojae*

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV - Ps049	202-2	Bioassay	Williams - L93-3312	2022	Santa Margarida do Sul	RS	101	-30.3385	-54.0707	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps050	274-4	Bioassay	Williams - L93-3312	2022	Guarapuava	PR	103	-25.3935	-51.4566	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps051	274-5	Bioassay	Williams - L93-3312	2022	Guarapuava	PR	103	-25.3935	-51.4566	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps052	199-2	Bioassay	Williams - L93-3312	2022	Caçapava do Sul	RS	101	-28.6394	-53.6062	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps053	210-4	Bioassay	Williams - L93-3312	2022	Alegrete	RS	101	-29.7848	-55.7757	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps054	207-4	Bioassay	Williams - L93-3312	2022	Dom Pedrito	RS	101	-30.9818	-54.6775	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps055	207-5	Bioassay	Williams - L93-3312	2022	Dom Pedrito	RS	101	-30.9818	-54.6775	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps056	184-1a	Bioassay	Williams - L93-3312	2022	São Borja	RS	101	-28.6829	-55.9775	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps057	184-1b	Bioassay	Williams - L93-3312	2022	São Borja	RS	101	-28.6829	-55.9775	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps058	184-2	Bioassay	Williams - L93-3312	2022	São Borja	RS	101	-28.6829	-55.9775	Phytophthora	Pathogenic	*Phytophthora sojae*

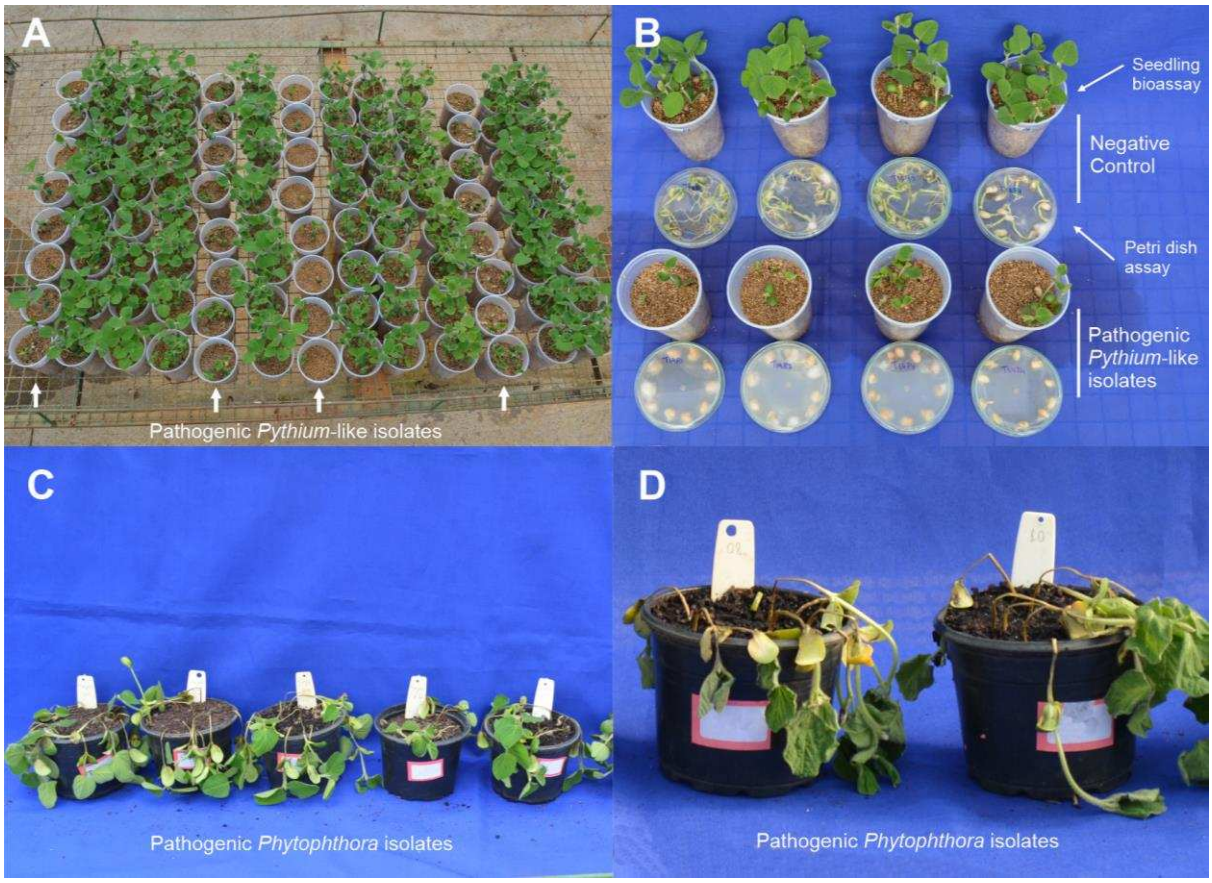
ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV - Ps059	184-3	Bioassay	Williams - L93-3312	2022	São Borja	RS	101	-28.6829	-55.9775	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps060	172-1	Bioassay	Williams - L93-3312	2022	Bela Vista	MS	204	-22.0825	-56.5259	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps061	190-1	Bioassay	Williams - L93-3312	2022	Tapes	RS	101	-30.6742	-51.3966	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps062	190-2	Bioassay	Williams - L93-3312	2022	Tapes	RS	101	-30.6742	-51.3966	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps063	190-3	Bioassay	Williams - L93-3312	2022	Tapes	RS	101	-30.6742	-51.3966	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps064	087-1	Bioassay	Williams - L93-3312	2022	Londrina	PR	201	-23.2927	-51.1732	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps065	087-2	Bioassay	Williams - L93-3312	2022	Londrina	PR	201	-23.2927	-51.1732	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps066	128-1	Leaf-bait	Williams	2022	Londrina	PR	201	-23.2927	-51.1732	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps067	128-2	Leaf-bait	Williams	2022	Londrina	PR	201	-23.2927	-51.1732	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps068	128-3	Leaf-bait	Williams	2022	Londrina	PR	201	-23.2927	-51.1732	Phytophthora	Pathogenic	*Phytophthora sojae*

ID	Soil sample	Bait	Cultivar	Date	Municipality	State	ECRs[1]	Latitude	Longitude	Group	Pathogenicity	Species
UFV - Ps069	396	Bioassay	Williams - L93-3312	2023	Palotina	PR	201	-24.2817	-53.8404	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps070	396	Bioassay	Williams - L93-3312	2023	Palotina	PR	201	-24.2817	-53.8404	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps071	396	Bioassay	Williams - L93-3312	2023	Palotina	PR	201	-24.2817	-53.8404	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps072	396	Bioassay	Williams - L93-3312	2023	Palotina	PR	201	-24.2817	-53.8404	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps073	398	leaf-bait	Williams - L93-3312	2023	Candido Mota	SP	102	-22.7461	-50.3875	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps074	398	leaf-bait	Williams - L93-3312	2023	Candido Mota	SP	102	-22.7461	-50.3875	Phytophthora	Pathogenic	*Phytophthora sojae*
UFV - Ps075	172	leaf-bait	Williams - L93-3312	2023	Bela Vista	MS	204	-22.0825	-56.5259	Phytophthora	Pathogenic	*Phytophthora sojae*

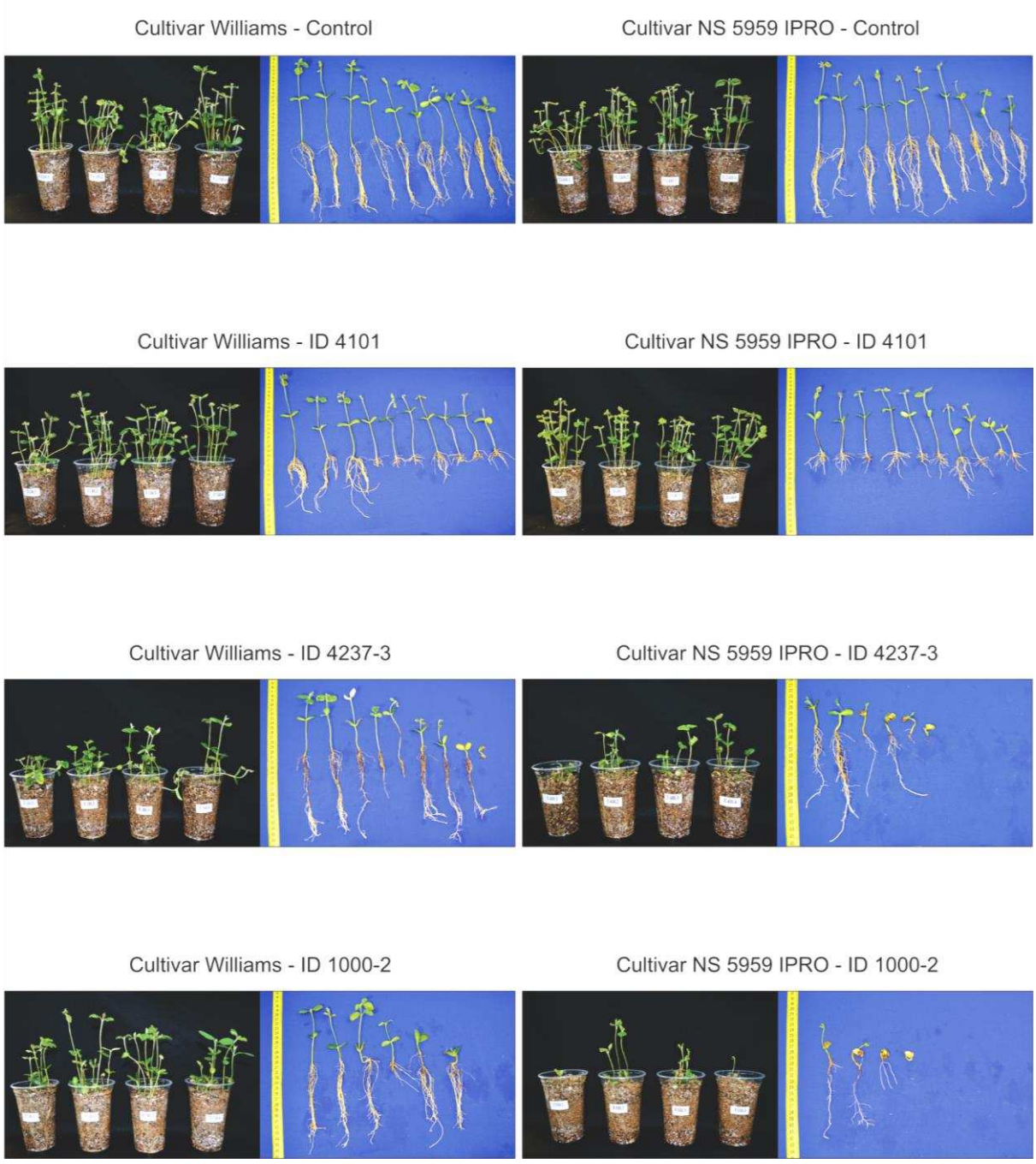
ECRs[1]: Soybean-producing edaphoclimatic microregions in Brazil; NT[2]: Isolate not tested.



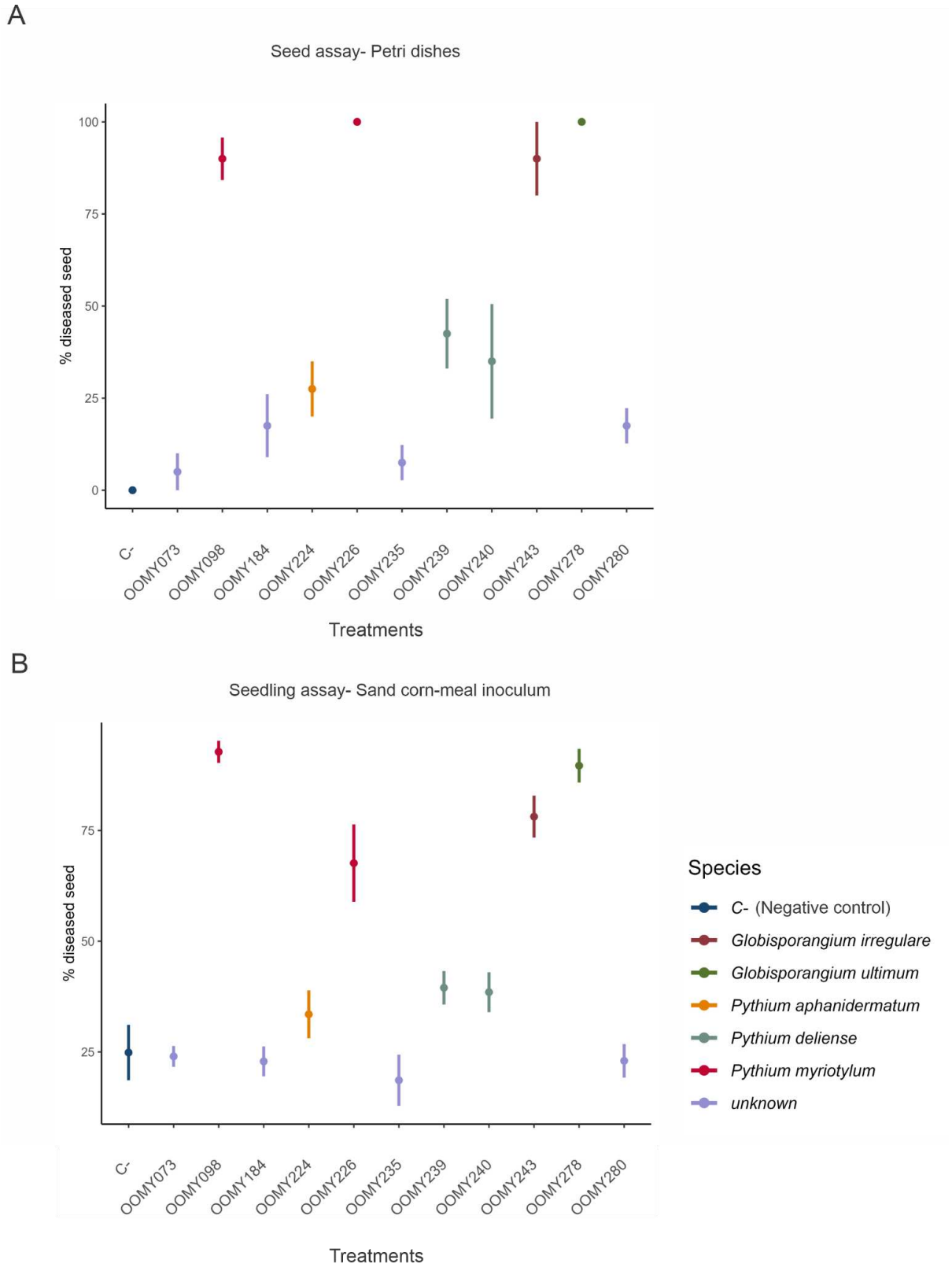
Supplementary Figure 1. Oomycete isolation methods. (A) modified soybean leaf baiting and (B) a bioassay technique. (C) Water-soaked lesions that developed on the leaflets tissues and (D) Necrosis and water-soaked lesions on the hypocotyl of the seedling.



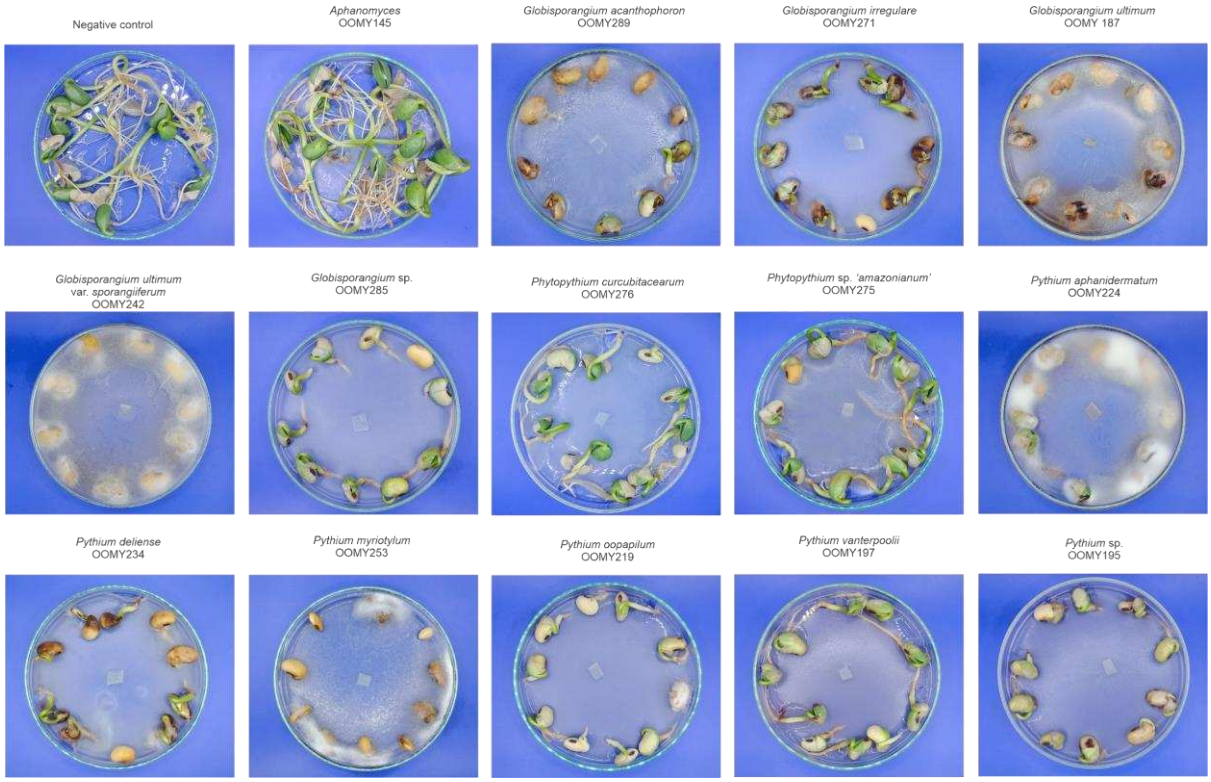
Supplementary Figure 2. Pathogenicity assays of Oomycete isolates. Assay with *Pythium*-like isolates (A). Comparison of the two assays of pathogenicity with *Pythium*-like isolates (B). Assay with *P. sojae* isolates (C and D).



Supplementary Figure 3. Sand-corn meal pathogenicity assay with *Pythium* isolates. Demonstration of the comparative susceptibility of both soybean cultivars. The two cultivars had similar symptoms when subjected to previous tests, indicating a uniform response to the pathogenicity assay.



Supplementary Figure 4. *Pythium* pathogenicity tests. (A) Petri dish assay and (B) seedling pathogenicity assay.



Supplementary Figure 5. Pathogenicity of Pythiaceous isolates in Petri dish assay.

CHAPTER 2 - A shift in pathotype diversity and complexity of *Phytophthora sojae* in Brazil

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Abstract

Soybean root and stem rot caused by the oomycete *Phytophthora sojae* is a destructive disease worldwide that can affect plants at any growth stage. The use of resistant cultivars is the most effective method of controlling the disease. Therefore, monitoring changes in the population of *P. sojae* regarding the dynamics of avirulence genes capable of overcoming resistance genes (*Rps*) is important to reduce yield losses and to enhance the effectiveness of the *Rps* genes. Forty isolates of *P. sojae* sampled from a region of high incidence of soybean root and stem rot in Brazil were characterized using 14 soybean differentials and 28 pathotypes were identified. Compared to a study conducted a decade ago there was a major shift in pathotype diversity and complexity towards both higher numbers of different pathotypes and of avirulence genes in a given individual in the current population of *P. sojae*. Breeding programs aiming at developing soybean cultivars with resistance to root and stem rot should consider the high variability in the population of *P. sojae* and seek for strategic deployment of genes and germplasm.

Keywords: Phytophthora Root Rot, soybean, *Glycines max*, races, Rps genes, resistance

1. Short communication content

Soybean root and stem rot (SRSR) caused by the oomycete *Phytophthora sojae* Kaufm. & Gerd can significantly reduce yield and the high economic losses associated with SRSR epidemics rank the disease among the top five of this crop in most soybean-producing countries (Kamoun et al. 2015). In Brazil, epidemics of SRSR occur more regularly in the South region and disease incidence is higher in areas planted every year with soybeans (Costamilan et al. 1996). The only survey of pathotypes of *P. sojae* in Brazil was carried out in 2013 in which 37 isolates were characterized and 17 pathotypes were reported (Costamilan et al. 2013). Over the past decade the number of soybean varieties recommended for planting in the South region increased and there is a "mosaic" of several cultivars being planted in the region. In fact, it is common practice to plant two or more cultivars in a single farm. Depending on the resistance genes to *P. sojae* (*Rps*) present in the soybean cultivars, such a diversified portfolio of commercial varieties may lead to variable selection pressure on the pathogen population.

Populations of *P. sojae* are genetically variable and the dynamics of the avirulence genes in the pathogen population is greatly influenced by the set of *Rps* genes present in the soybean cultivars planted. In Brazil, there is no formal and publicly available information regarding the utilization of *Rps* genes in commercial soybean cultivars. But, a careful inspection at the portfolios of soybean seed companies revealed that *Rps* 1k and 7 are the most commonly listed genes in soybean varieties planted in the South region. Thus, it is anticipated that the effectiveness of these genes may be reduced in areas intensively cultivated with soybean varieties carrying them. Under this scenario, an important action to extend the life of a resistant soybean cultivar is to monitor the pathotype dynamics by means

of periodical surveys (Costamilan et al. 2013; Stewart et al. 2011; McCoy et al. 2022). Thus, the objective of this work was to characterize the pathotype profile of *P. sojae* isolates obtained from the South region of Brazil during the 2020/21 growing season.

Soybean seedling damping-off and root rot were observed in different municipalities in Rio Grande do Sul, Santa Catarina and Paraná states, Brazil. Soil samples from areas with reported occurrence of SRSR (N=5) and also from areas of suspected disease occurrence (N=11) were collected in a zigzag pattern taking 10 to 15 subsamples to amount to a total of 1500 g of soil in each area. In the laboratory, each soil sample was homogenized and pathogen isolation was attempted using a modified leaf baiting technique and a bioassay without prior preparation for oospore germination. For leaf baiting, 200 g of each soil sample was distributed as homogeneously as possible into a 1500 mL plastic container (198 mm length x 152 mm width x 86 mm height) and flooded with 500 mL of distilled water such as to provide a 2 cm-high water column above the top of the soil layer. After 24 h under room temperature, trifoliate leaves of the *P. sojae*-susceptible cultivar Williams were placed afloat on the water surface and the containers were kept at 23 oC for up to 7 days. Water-soaked lesions that developed on the leaf tissues were inspected under a stereomicroscope and those with reproduction structures resembling *P. sojae* were used for pathogen isolation using the methods described below.

For the bioassay method, 20 to 30 seeds of 'Williams' were distributed on the surface of infested soil samples placed in 10 cm-diameter pots and covered with a wet layer of organic planting substrate (HF - Mecplant). After seed germination, the pots were flooded at every 48 h until seedlings with damping-off and water-soaked lesions on the hypocotyl were observed. Water-soaked lesions formed on the leaves (from the leaf baiting method) or in the hypocotyl were excised using a scalpel, plated

on *Phytophthora* selective medium (PBNIC) (Dorrance et al. 2008) and incubated at 25 ºC and 12 h-photoperiod. Cultures were characterized as *P. sojae* by observing morphological traits and by conventional PCR amplification using the *P. sojae*-specific primers PSOJF1 and PSOJR1 (Bienapfl et al. 2011).

Forty isolates of *P. sojae* were obtained from 15 soybean fields distributed in 11 municipalities (Table 1). Pure cultures were transferred to a slant of carrot-agar for storage at 18 ºC. Pathotypes of *P. sojae* isolates were determined using the hypocotyl technique (Kaufmann and Gerdemann 1958) on a set of 14 differential soybean genotypes: L88-8470 (*Rps*1a), L77-1863 (*Rps*1b), L75-3735 (*Rps*1c), L93-3312 (*Rps*1d), L77-1794 (*Rps*1k), L76-1988 (*Rps*2), L83-570 (*Rps*3a), L89-1541 (*Rps*3b), L92-7857 (*Rps*3c), L85-2352 (*Rps*4), L85-3059 (*Rps*5), L89-1581 (*Rps*6), L93-3258 (*Rps*7), PI 399073 (*Rps*8), and cultivar Williams (*rps*) as susceptible. Ten seedlings (fifteen-day-old) of each differential were inoculated by injecting approximately 0.1 mL of mycelial slurry of each isolate into the hypocotyl. The isolate Ps36.1 of *P. sojae* (virulence formula 1b, 1d, 3a, 3b, 3c, 4, 5, 6, 7 and 8, pathotype 21773) (Costamilan et al. 2022) was used as a positive control in this assay. The plants were incubated in a dew chamber at 24 ºC for 24 h and then placed in a greenhouse at 24 ºC for 5 days when they were assessed. The number of dead seedlings were recorded and to score the reaction as susceptible at least seven seedlings should be dead; i.e. a cutoff value of 70% susceptibility (Stewart et al. 2016; Grijalba et al. 2020; Matthiesen et al. 2021; Hebb et al. 2022). The distribution of susceptible reactions, pathotype complexity, pathotype frequency, and diversity indices were estimated using the HaGiS package in R (McCoy et al 2019) and the octal nomenclature was used to summarize pathotype descriptions as previously described (Dorrance et al. 2003).

Table 1. Isolates of *Phytophthora sojae* from different soybean-producing areas in the South region of Brazil, and pathotype characterization.

ID		Disease occurrence	Isolation method	Location				Pathotype Characterization		
Isolate	sample			Municipality	State	Latitude	Longitude	Pathotype	Complexities	Octal
UFV - Ps001	286	Yes	Leaf-bait	Não Me toque	RS	-28.4579	-52.8220	1b, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7	10	27571
UFV - Ps002	286	Yes	Leaf-bait	Não Me toque	RS	-28.4579	-52.8220	1b, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	11	27573
UFV - Ps003	287	Yes	Leaf-bait	Não Me toque	RS	-28.4579	-52.8220	1b, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	11	27573
UFV - Ps004	287	Yes	Leaf-bait	Não Me toque	RS	-28.4579	-52.8220	1b, 1d, 1k, 2, 3a, 3b, 3c, 5, 6, 7, 8	11	27763
UFV - Ps005	340	Yes	Leaf-bait	Sarandi	RS	-27.9548	-52.9152	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	12	27773
UFV - Ps006	286	Yes	Leaf-bait	Não Me toque	RS	-28.4579	-52.8220	1b, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	11	27573
UFV - Ps007	188	Yes	Leaf-bait	Tapes	RS	-30.6742	-51.3966	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573
UFV - Ps008	341	Yes	Leaf-bait	Sarandi	RS	-27.9548	-52.9152	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	12	27773
UFV - Ps009	186	Yes	Leaf-bait	Tapes	RS	-30.6742	-51.3966	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573
UFV - Ps010	341	Yes	Leaf-bait	Sarandi	RS	-27.9548	-52.9152	1d, 1k, 2, 3a, 4, 5, 6, 7	8	07171
UFV - Ps011	334	Suspected	Leaf-bait	Cruz Alta	RS	-28.6394	-53.6062	1c, 1d, 6, 8	4	41042

ID		Disease occurrence	Isolation method	Location				Pathotype Characterization		
Isolate	sample			Municipality	State	Latitude	Longitude	Pathotype	Complexities	Octal
UFV - Ps012	334	Suspected	Leaf-bait	Cruz Alta	RS	-28.6394	-53.6062	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	13	77573
UFV - Ps013	322	Suspected	Leaf-bait	Abelardo Luz	SC	-26.5672	-52.3339	1b, 1d, 1k, 2, 3a, 3c, 4, 6, 7	9	27551
UFV - Ps014	322	Suspected	Leaf-bait	Abelardo Luz	SC	-26.5672	-52.3339	1b, 1d, 1k, 2, 3c, 5, 6	7	27460
UFV - Ps015	325	Suspected	Leaf-bait	Cruz Alta	RS	-28.6394	-53.6062	1d, 2	2	05000
UFV - Ps016	325	Suspected	Leaf-bait	Cruz Alta	RS	-28.6394	-53.6062	1d, 2, 4, 5, 6	5	05070
UFV - Ps017	325	Suspected	Leaf-bait	Cruz Alta	RS	-28.6394	-53.6062	1d, 4	2	01010
UFV - Ps018	318	Suspected	Leaf-bait	Cruz Alta	RS	-28.6394	-53.6062	1a, 5, 6	3	10060
UFV - Ps020	220	Suspected	Bioassay	Santo Augusto	RS	-27.8515	-53.7772	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	11	27771
UFV - Ps021	220	Suspected	Bioassay	Santo Augusto	RS	-27.8515	-53.7772	1b, 1d, 1k, 2, 3a, 3b, 6, 7	8	27341
UFV - Ps022	221	Suspected	Bioassay	Santo Augusto	RS	-27.8515	-53.7772	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	11	27771
UFV - Ps023	221	Suspected	Bioassay	Santo Augusto	RS	-27.8515	-53.7772	1b, 1d, 1k, 2, 3a, 3b, 3c, 6, 7	9	27741
UFV - Ps024	340	Yes	Bioassay	Sarandi	RS	-28.4579	-52.8220	1b, 1d, 1k, 2, 3a, 3b, 3c, 6, 7	9	27741
UFV - Ps025	340	Yes	Bioassay	Sarandi	RS	-28.4579	-52.8220	1b, 1d, 1k, 2, 3a, 3b, 4, 5, 6, 7	10	27371

ID		Disease occurrence	Isolation method	Location				Pathotype Characterization		
Isolate	sample			Municipality	State	Latitude	Longitude	Pathotype	Complexities	Octal
UFV - Ps026	210	Suspected	Bioassay	Alegrete	RS	-29.7848	-55.7757	1d, 2, 3a, 3c, 4, 5, 6, 7, 8	9	05573
UFV - Ps027	210	Suspected	Bioassay	Alegrete	RS	-29.7848	-55.7757	1b, 1d, 2, 3a, 7, 8	6	25103
UFV - Ps028	220	Suspected	Bioassay	Santo Augusto	RS	-27.8515	-53.7772	1b, 1d, 1k, 2, 3a, 3b, 5, 6, 7	9	27361
UFV - Ps030	272	Suspected	Bioassay	Guarapuava	PR	-25.3935	-51.4566	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	13	77573
UFV - Ps031	272	Suspected	Bioassay	Guarapuava	PR	-25.3935	-51.4566	1a, 1b, 1c, 1d, 1k, 2, 6	7	77040
UFV - Ps032	272	Suspected	Bioassay	Guarapuava	PR	-25.3935	-51.4566	1a, 1b, 1c, 1d, 1k, 2, 3a, 4, 5, 6, 7	11	77171
UFV - Ps033	222	Suspected	Bioassay	Senador Salgado Filho	RS	-28.6394	-53.6062	1b, 1d, 2, 3a, 3c, 4, 8	7	25512
UFV - Ps035	222	Suspected	Bioassay	Senador Salgado Filho	RS	-28.6394	-53.6062	1b, 1d, 3a	3	21100
UFV - Ps036	199	Suspected	Bioassay	Caçapava do Sul	RS	-30.5164	-53.4868	1b, 1d, 2, 3a, 3c, 5, 6	7	25560
UFV - Ps037	200	Suspected	Bioassay	Caçapava do Sul	RS	-30.5164	-53.4868	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	13	77573
UFV - Ps039	200	Suspected	Bioassay	Caçapava do Sul	RS	-30.5164	-53.4868	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	13	77573
UFV - Ps042	207	Suspected	Bioassay	Dom Pedrito	RS	-30.9818	-54.6775	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573
UFV - Ps045	222	Suspected	Bioassay	Senador Salgado Filho	RS	-28.6394	-53.6062	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573

ID		Disease occurrence	Isolation method	Location				Pathotype Characterization		
Isolate	sample			Municipality	State	Latitude	Longitude	Pathotype	Complexities	Octal
UFV - Ps046	222	Suspected	Bioassay	Senador Salgado Filho	RS	-28.6394	-53.6062	1b, 1d, 2, 3a, 3c, 5, 6, 7, 8	9	25563

A total of 28 pathotypes out of 40 *P. sojae* isolates were identified. The most frequent pathotype detected were 25573 (virulence formula: 1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8) and 77573 (1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8) each representing 10% of the isolates (N=4), followed by the pathotype 27573 (1b, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8) corresponding to 7.50% of the isolates, N=3), and the pathotypes 27771 (1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7), 27773 (1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8), 27741 (1b, 1d, 1k, 2, 3a, 3b, 3c, 6, 7), and 01010 (1d, 4), all corresponding to 5% of the isolates (N=2). The other 52.5% (21 isolates) were unique pathotypes. All *P. sojae* isolates were virulent on *Rps*1d (Table 1). Over 60% of the isolates were virulent on *Rps*1b, *Rps*2, *Rps*3a, *Rps*3c, *Rps*4, *Rps*5, *Rps*6 and *Rps*7. Twenty-three isolates were virulent to *Rps*1k (58.9%), 19 isolates (48.7%) were virulent to *Rps*8, while only seven isolates (17.50%) were virulent on *Rps*1a and *Rps*1c (Figure 1).

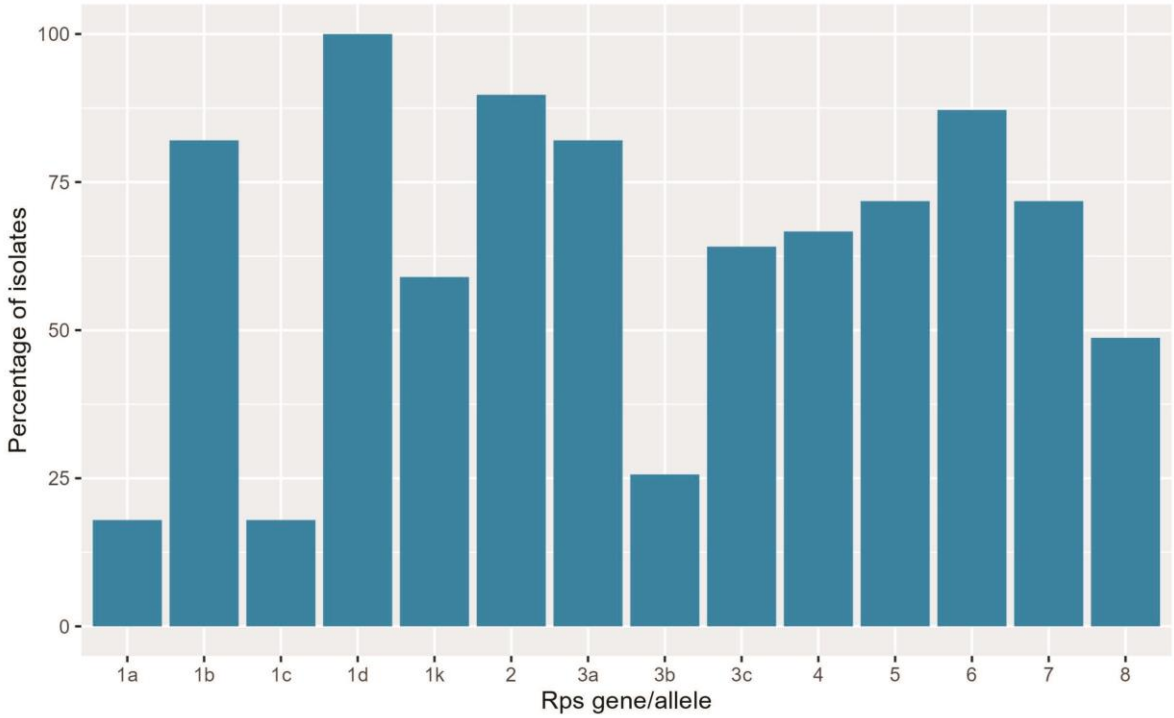

Figure 1. Percentage of *Phytophthora sojae* isolates recovered in a survey conducted in soybean fields in the South region of Brazil in the 2020/2021 crop season that were virulent on a specific *Rps* gene/allele.

The *Rps*2 gene has an intermediate reaction when *P. sojae* is inoculated in the hypocotyl, but the results with this gene were considered in the current study to allow comparisons with other findings (Mideros et al. 2007; Chowdhury et al. 2021; McCoy et al. 2022).

The percentage of isolates capable to overcome *Rps4* and *Rps6* genes was 66.66% and 87.17%, respectively. Because *Avr*4/6 of *P. sojae* is a single gene that interacts with both *Rps*4 and *Rps*6 genes (Dou et al. 2010), one would anticipate the same percentage of isolates scoring susceptible reactions in the differentials carrying *Rps4* and *Rps6* genes. The N- terminal of Avr4/6 triggers Rps4-dependent HR, while the C-terminal triggers Rps6-mediated HR, and differences in interaction of *Rps4* or *Rps6* with both domains of *Avr4/6* may explain the variation in response to an isolate (Whisson et al 2004; Dou et al. 2010). Variation in susceptible interactions with *Rps*4 and *Rps*6 genes such as the one observed in the present study was also observed in other studies that used the same inoculation method and a similar set of differentials (Kaitany et al. 2001; Cui et al. 2010; Costamilan et al. 2013; Chowdhury et al. 2021; McCoy et al. 2022; Hebb et al. 2022).

All *Rps* genes were overcome by at least one isolate of *P. sojae* (Figure 1). The mean number of resistance genes that a given pathotype could overcome; i.e. pathotype complexity, was 8.62. The pathotype complexity reported by Costamilan et al. (2013) for 37 isolates sampled from the same region (South) was 6.70. The calculated diversity indices for the current population of *P. sojae* in the South region were: Simple diversity = 0.70; Gleason = 7.31; Shannon = 3.19; Simpson diversity = 0.95; and Evenness = 0.95. All these indices are known to be sample size-dependent. Therefore, even though the sample sizes of the two studies conducted to date to estimate diversity of pathotypes of *P. sojae* in Brazil are similar, Hill numbers for the

three orders of diversity measures; q = 0, species richness; q = 1, the exponential of Shannon's entropy index; and q = 2, the inverse of Simpson's concentration index were calculated and confidence bands were estimated to allow appropriate comparisons (Chao et al. 2014). The non-overlapping confidence intervals of point estimates of all orders of q (0, 1 and 2) suggest that the diversity estimates of the current population of *P. sojae* in Brazil has significantly increased over a 10-year period (Figure 3).

It is worth noting that Costamilan et al. (2013) used a cutoff = 80% of susceptibility for determining pathotype composition, while in this study the cutoff value was 70% because it best represented the interactions of the differentials and also because this is the most often used value in *P. sojae* pathotype diversity studies. In order to explore the effects of variable cutoff values on the diversity indices a comparative analysis was conducted with the data from the current study assuming three susceptibility cutoff values: 60%, 70% and 80% (Table S1). Regardless of the cutoff values, the diversity indices in the current population of *P. sojae* were higher than those estimated in the 2013 study (Table S1).

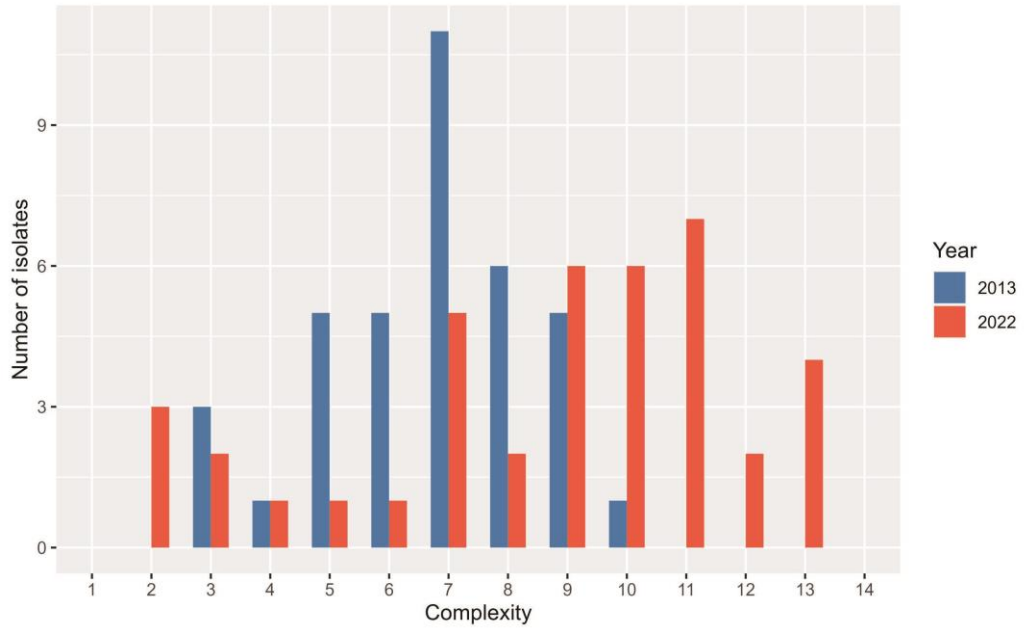

Figure 2. Number of isolates of *Phytophthora sojae* that were capable of overcoming different *Rps* genes (Complexity). Isolates obtained from the South region of Brazil in the 2020/2021 season are represented in red while those from the study by Costamilan et al. (2013) are represented in blue.

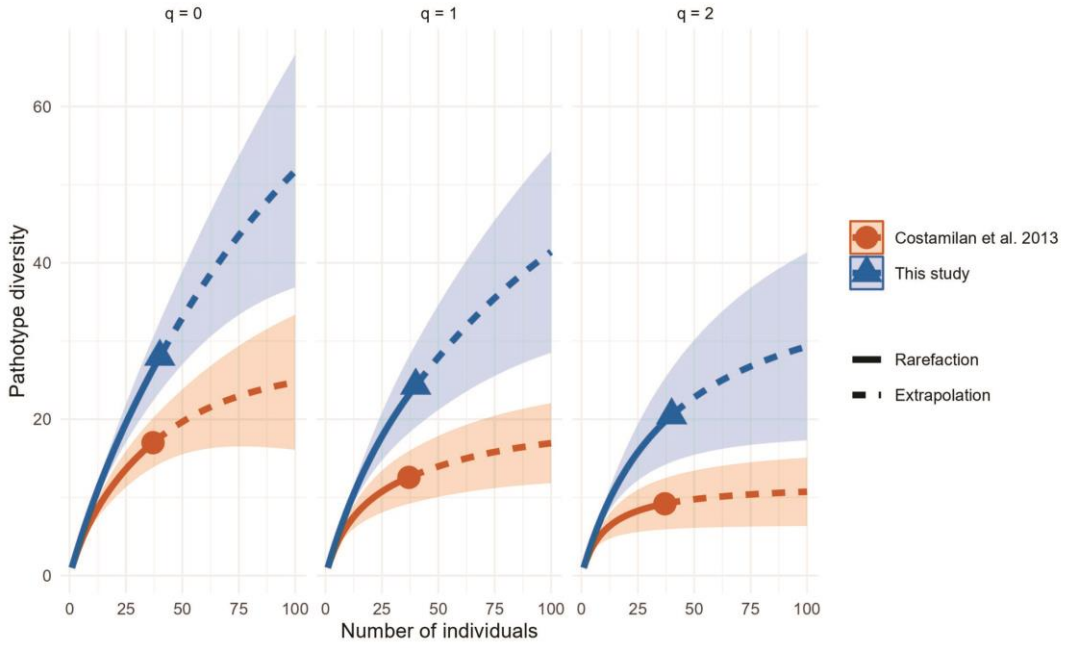

Figure 3. Rarefaction curves based on Hill numbers of orders q = 0 (richness), 1 (exponential of Shannon's entropy index) and 2 (inverse of Simpson's concentration index) used to estimate diversity of pathotypes of *Phytophthora sojae* from the study by Costamilan et al. (2013) (N = 37) and from this study (2020/21; N = 40). Interpolation (solid), extrapolation (dashed) lines and their 95% confidence intervals (shaded area) are indicated.

Compared to the results of five studies conducted during the past two decades in North America (Kaitany et al., 2001; Stewart et al., 2016; Chowdhury et al., 2021; Matthiesen et al., 2021; McCoy et al., 2022; and Hebb et al., 2022), the Brazilian population of *P. sojae* seems to be more variable, with higher complexity, and the changes in the avirulence gene pool seem to occur at a faster pace. This scenario imposes the need for special attention of the breeding programs aimed at controlling SRSR in Brazil, as well as for the strategic deployment of the resistance genes currently known and largely used by seed companies.

Acknowledgements

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The authors declare that they have no conflict of interest.

2. References

Bienapfl, J. C., Malvick, D. K., and Percich, J. A. 2011. Specific molecular detection of *Phytophthora sojae* using conventional and real-time PCR. Fungal Biol. 115:733–740.

Chao, A., Chiu, C. H., and Jost, L. 2014. Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through hill numbers. Annu. Rev. Ecol. Evol. Syst. 45:297–324.

Chowdhury, R. N., Tande, C., and Byamukama, E. 2021. Common Phytophthora Sojae Pathotypes Occurring In South Dakota. Plant Heal. Prog. 22:1–6.

Costamilan, L. M., Bonato, E. R., Urben, A. F., Matsuoka, K., and Vanetti, C. A. 1996. Occurrence of *Phytophthora sojae* in Brazil. Fitopatol. Bras. 21:395.

Costamilan, L. M., Clebsch, C. C., Soares, R. M., Seixas, C. D. S., Godoy, C. V., and Dorrance, A. E. 2013. Pathogenic diversity of *Phytophthora sojae* pathotypes from Brazil. Eur. J. Plant Pathol. 135:845–853.

Costamilan, L. M., Bertagnolli, P. F., Melo, C. L. P. de, Soares, R. M., and Clebsch, C. C. 2022. Resistência a *Phytophthora sojae* em linhagens de soja da Embrapa, em 2021. - Portal Embrapa.

Cui, L., Yin, W., Tang, Q., Dong, S., Zheng, X., Zhang, Z., Wang, Y. 2010. Distribution, pathotypes, and metalaxyl sensitivity of *Phytophthora sojae* from Heilongjiang and Fujian provinces in China.

Dorrance, A. E., McClure, S. A., and DeSilva, A. 2003. Pathogenic diversity of *Phytophthora sojae* in Ohio soybean fields. Plant Dis. 87:139–146.

Dorrance, A. E., Berry, S. A., Anderson, T. R., and Meharg, C. 2008. Isolation, storage, pathotype characterization, and evaluation of resistance for *Phytophthora sojae* in soybean. Plant Health Prog. 9:1.

Dou, D., Kale, S. D., Liu, T., Tang, Q., Wang, X., Arredondo, F. D., Basnayake, S., Whisson, S., Drenth, A., Maclean, D., & Tyler, B. M. 2010. Different domains of *Phytophthora sojae* effector Avr4/6 Are recognized by soybean resistance genes *Rps*4 and *Rps*6. Mol. Plant- Microbe Interact. 23:425–435.

Garcia, D. G. C., Huang, S.-Y., Kleczewski, N. M., and Mideros, S. 2022. Virulence, aggressiveness, and fungicide sensitivity of *Phytophthora* spp. associated with soybean in Illinois. Plant Dis.

Grijalba, P. E., Martínez, M. C., and Guillin, E. 2020. Pathotype and SSR variation in *Phytophthora sojae* from the Argentinean Pampas. J. Phytopathol. 168:228–243.

Hebb, L. M., Bradley, C. A., Mideros, S. X., Telenko, D. E. P., Wise, K., and Dorrance, A. E. 2022. Pathotype complexity and genetic characterization of *Phytophthora sojae p*opulations in Illinois, Indiana, Kentucky, and Ohio. Phytopathology. 112:663–681.

Kaitany, R. C., Hart, L. P., and Safir, G. R. 2001. Virulence composition of *Phytophthora sojae* in Michigan. Plant Dis. 85:1103–1106.

Kamoun, S., Furzer, O., Jones, J. D. G., Judelson, H. S., Ali, G. S., Dalio, R. J. D., Schena, L., Zambounis, A., Panabières, F., Cahill, D., Ruocco, M., Figueiredo, A., Chen,X. R., Hulvey, J., Stam, R., Lamour, K., Gijzen, M., Tyler, B. M., Grünwald, N. J., Mukhtar, S., Tomé, D. F. A., Tör, M., Ackerveken, G. V. D., Mcdowell, J., Daayf, F., Fry, W. E., Lindqvist- Kreuze, H., Meijer, B. P., Ristaino, J., Yoshida, K., Birch, P. R. J. and Govers, F. 2015. The top 10 oomycete pathogens in molecular plant pathology. Mol. Plant Pathol. 16:413–434.

Kaufmann, M. J. and Gerdemann, J. W. 1958. Root and stem rot of soybean caused by *Phytophthora sojae* n.sp. Phytopathology 48:201–208.

Matthiesen, R. L., Schmidt, C., and Robertson, A. E. 2021. Comparison of baiting techniques for recovering *Phytophthora sojae* from soybean fields in Iowa. Plant Heal. Prog. 22:316– 322.

McCoy, A. G., Noel, Z., Sparks, A. H., and Chilvers, M. 2019. hagis, an R package resource for pathotype analysis of *Phytophthora sojae* populations causing stem and root rot of soybean. Mol. Plant-Microbe Interact. 32:1574–1576.

McCoy, A. G., Noel, Z. A., Jacobs, J. L., Clouse, K. M., and Chilvers, M. I. 2022. *Phytophthora sojae* pathotype distribution and fungicide sensitivity in Michigan. Plant Dis. 106:425–431.

Mideros, S., Nita, M., and Dorrance, A. E. 2007. Characterization of components of partial resistance, *Rps*2, and root resistance to *Phytophthora sojae* in soybean. Phytopathology 97:655–662.

Stewart, S., Wickramasinghe, D., Dorrance, A. E., and Robertson, A. E. 2011. Comparison of three microsatellite analysis methods for detecting genetic diversity in *Phytophthora sojae* (Stramenopila: Oomycete). Biotechnol. Lett. 33:2217–2223.

Stewart, S., Robertson, A. E., Wickramasinghe, D., Draper, M. A., Michel, A., and Dorrance, A. E. 2016. Population structure among and within Iowa, Missouri, Ohio, and South Dakota populations of *Phytophthora sojae*. Plant Dis. 100:367–379.

Whisson, S. C., Basnayake, S., Maclean, D. J., Irwin, J. A. G., and Drenth, A. 2004. *Phytophthora sojae* avirulence genes *Avr*4 and *Avr*6 are located in a 24 kb, recombination- rich region of genomic DNA. Fungal Genet. Biol. 41:62–74.

Supplementary data

CHAPTER 2 - A shift in pathotype diversity and complexity of *Phytophthora sojae* in Brazil

Authors: I. C. A. Batista, M. P. C. Silva, A. L. Silva Junior, M. P. Gonzalez, M. P. Camargo, A. Figueiredo, B. T. Hora Junior, E. S. G. Mizubuti.

Supplementary Table 1. Number of pathotypes, mean complexity of the pathotype and diversity indices of *Phytophthora sojae* isolates from different studies.

Reference	**This study[1]**			**Costamilan et al. 2013**	**Stewart et al. 2014**	**Stewart et al. 2016 Iowa / Ohio / South Dakota**	**Grijalba et al. 2020[2]**	**Chowdhury et al. 2021**	**Matthiesen et al. 2021 (Iowa / Nebraska)**	**Kaitany et al. 2001**	**McCoy et al. 2022**	**Hebb et al. 2022 (Indiana, Kentucky, Ohio / Illinois)**
Cutoff	**60%**	**70%**	**80%**	**80%**	**70%**	**70%**	**70%**	**60%**	**70%**	**60%**	**60%**	**70%**
Number of samples	40	40	40	37	121	17 / 33 / 20	92	171	258 / 68	78	83	532 / 5
Pathotypes	27	28	35	17	14	10 / 30 / 17	34	48	15 / 10	57	53	218 / 10
Mean complexity	9.65	8.625	7.625	6.70	-	3.7 / 5.7 / 4.6	-	5.1	5.5 / 6.5	7.2	9.2	-
Simple index	0.67	0.7	0.875	0.45	0.11	0.58 / 0,90 / 0.85	0.36	0.28	0.06 / 0.15	0.73	0.63	0.46 / 0.71
Gleason index	7.04	7.31	9.21	4.43	2.71	0.25 / 8,29 / 5,34	7.29	9.14	2.52 / 2.13	12.85	11.76	35.23 / 3.41
Shannon index	3.15	3.19	3.50	2.53	-	2.15 / 3.37 / 2.76	3.21	-	2.04 / 1.64	3.90	3.76	4.64 / 2.24
Simpson index	0.95	0.95	0.96	0.89	-	-	0.94	-	0.83 / 0.71	-	-	0.97 / 0.97
Evenness	0.95	0.95	0.98	0.89	-	-	0.91	-	0.75 / 0.71	-	-	-

[1]We performed a comparative analysis with data from the present study assuming three different susceptibility cutoff values: 60%, 70% and 80%.
[2]Grijalba et al. 2020 used a different set of cultivars to determine the pathotypes, with 8 genotypes and two susceptible cultivars.

CHAPTER 3 - Mapping pathotype variability and genetics of *Phytophthora sojae* in Brazil

I. C. A. Batista[1], L. M. Costamilan[2], A. L. Silva Junior[1], A. F. Silva[1], G. C. F. de Melo[1], and E. S. G. Mizubuti[1]†

[1]Departamento de Fitopatologia, Universidade Federal de Viçosa, MG, 36570-900, Brazil.
[2]Embrapa Trigo, RS, 99050-970, Brazil.

†Corresponding author: E.S.G. Mizubuti; E-mail: mizubuti@ufv.br

Abstract

This study investigates the genetic diversity and temporal dynamics of *P. sojae* populations in Brazilian soybean-producing regions. Samples were collected from soybean fields across five states in Brazil over a decade. The isolates were genotyped using microsatellite markers (SSR), and pathotypes were determined based on the responses on soybean differential lines carrying resistance genes. The study identified 37 unique pathotypes in 2023, reflecting an increase in diversity and complexity compared to 2013. Genotypic analysis revealed high genetic diversity with no dominant lineages. There is limited differentiation among populations, emphasizing the consistency in genetic patterns, but there was no major change in the the structure of the population over time. Contrary to expectations, *P. sojae* populations in Brazil exhibited stability in genetic diversity and pathotype complexity over a decade. The observed changes in pathotypes indicate an ongoing arms race between the pathogen and soybean resistance genes. These findings contribute to understanding the evolutionary dynamics of *P. sojae*, guiding future strategies for sustainable soybean production in Brazil.

Keywords: Phytophthora Root Rot, soybean root rot, population genetics

1. Introduction

Soybean root and stem rot (SRSR) is a devastating plant disease caused by the oomycete *Phytophthora sojae* Kaufm. & Gerd and has been a concern to Brazilian growers, breeders, and pathologists. *P. sojae* can seriously damage soybean crops, one of main agricultural commodities in Brazil (Faostat 2023), and the understanding of the pathogen population requires continuous research efforts, mainly those studies related to changes in the pathotypes (phenotypes) and genotypes of the pathogen over time and the implications of these variants for yield losses.

The major strategy to manage SRSR is planting cultivars that have resistance to *P. sojae* genes (*Rps*). Therefore, monitoring the genetic variability of the pathogen is important to deploy effective cultivars and to ensure the durability of resistance genes, and also to determine future breeding efforts. To date, over 40 *Rps* genes or alleles have been documented on a global scale (Lin et al. 2022). In contrast, an increase in the diversity of pathotypes of *P. sojae* isolates is occurring over time in different countries, such as Argentina, Brazil, Canada, and China (Batista et al. 2023; McCoy et al. 2023). The SRSR continues to pose an ongoing challenge to soybean crops, due to the genetic diversity of the pathogen. This underscores the need to explore and acquire new sources of resistance.

The identification and characterization of pathotypes of *P. sojae* have been critical in guiding genetic resistance strategies and refining control practices. In Brazil, the first assessment of the diversity of *P. sojae* was conducted by Costamilan et al. (2013). Based on the results of this study steering efforts in breeding programs that considered SRSR resistance could be improved. The isolates of *P. sojae* used in the study were collected between 2006 and 2010, setting a foundation for subsequent research (Costamilan et al. 2013). A second study was recently conducted using

isolates of *P. sojae* collected between 2020 and 2022 (Batista et al., 2023). The major objective was to assess the current status of the pathotypes present in Brazil. A comparative analysis of the populations over a ten-year interval in the same soybean-crop areas located in the Central-South region of Brazil, revealed a shift in the diversity and complexity of *P. sojae* pathotypes. There was an increase of different pathotypes, which may result in a loss of efficiency of some *Rps* genes currently used for the management of SRSR (Batista et al. 2023).

The high variability detected in both studies carried out in Brazil (Costamilan et al. 2013; Batista et al. 2023) was somewhat counterintuitive in relation to the characteristics of *P. sojae*. As a soilborne pathogen, *P. sojae* has limited means of dispersal. Therefore, it is expected that gene flow among populations is restricted. Furthermore, *P. sojae* is homothallic and the populations display low allelic diversity with a few dominant genotypes prevailing at high frequencies (Förster et al. 1994; Gijzen and Qutob, 2009; McCoy et al. 2023). This characteristic contrasts with sexually outcrossing organisms, which frequently exhibit greater genotypic diversity. Despite the limitation in dispersal and the homothallic nature of *P. sojae,* the diversity of pathotypes observed in the population of this pathogen is noteworthy (Stewart et al. 2016).

The Brazilian population of *P. sojae* has not been genotyped with neutral markers. A set of simple sequence repeat (SSR) markers was developed and has been used to assess genetic diversity of *P. sojae* populations in the United States and Argentina (Dorrance and Grunwald 2008; Stewart et al. 2016; Grijalba et al. 2020). Quantifying the genetic diversity of the pathogen population and determining how variants are distributed in space may greatly contribute to enhance our understanding about the genetic and evolutionary dynamics of the population of *P. sojae* in Brazil.

The present work was conducted to test the hypothesis that the population of *P. sojae* undergoes significant variation over time and is geographically structured. Given that *P. sojae* is a soilborne homothallic microorganism, there might be a constraint on the genetic diversity within the population during the growing seasons. The objective of this study is to unravel the genetic and evolutionary aspects of the *P. sojae* population in Brazil, mainly to elucidate the mechanisms underpinning the evolution of virulence and the adaptive strategies of this pathogen.

2. Material and methods

2.1. Sampling

The sampling spanned soybean fields across five states located in different regions of Brazil: Mato Grosso do Sul (Central region), São Paulo (Southeast region), Paraná, Santa Catarina, and Rio Grande do Sul (South region). Field visits to collect soil samples were conducted on locations with reported or suspected occurrences of SRSR. These fields were chosen to represent the soybean edaphoclimatic macro-regions 1 and 2 (ECRs). These ECRs encompass geographical areas characterized by similar soil and climate conditions that are conducive to successful soybean cultivation (Zdziarski et al. 2018). Although the same ECRs were sampled from 2006 to 2022, individual sites were not the same and the sampling strategy was also slightly different. The details regarding sampling of the 2006 to 2012isolates can be found elsewhere (Costamilan et al. 2013).

2.2. Isolates of *P. sojae*

For the isolation of *P. sojae* from soil samples, standard procedures were applied as described in a previous study (Batista et al. 2023). All *P. sojae* isolates

obtained between 2020 to 2022 underwent purification to achieve monozoosporic colonies and were subsequently maintained in carrot-agar (CA). The isolates used in the study by Costamilan et al. (2013) were provided by Embrapa Trigo Passo Fundo, RS, Brazil. Altogether 120 isolates were used for the subsequent analysis (Table 1).

2.3. Identification of pathotypes

The pathotype of each *P. sojae* isolate was assessed on 14 differentially resistant lines of soybean carrying a single resistance gene or allele: L88-8470 (Rps1a), L77-1863 (Rps1b), L75-3735 (Rps1c), L93-3312 (Rps1d), L77-1794 (Rps1k), L76-1988 (Rps2), L83-570 (Rps3a), L89-1541 (Rps3b), L92-7857 (Rps3c), L85-2352 (Rps4), L85-3059 (Rps5), L89-1581 (Rps6), L93-3258 (Rps7), PI 399073 (Rps8), and the cultivar Williams (rps) as a susceptible line.

The susceptibility was determined by inoculating plantlets and then assessing the infection response five days later. All assays were performed twice, and any trials resulting in ambiguous findings were repeated. Pathotype assignment was based on the distinct responses of the differential lines to individual pathogen isolates, representing a unique combination of resistant and susceptible reactions.

The classification of pathotypes was based on the number of dead seedlings. The cutoff value used for classifying a soybean genotype as resistant or susceptible as 70% (Stewart et al. 2016; Grijalba et al. 2020; Matthiesen et al. 2021; Hebb et al. 2022). A resistant genotype had less than seven dead seedlings at the assessment time; while when more than seven seedlings were dead the reaction was considered as susceptible. The isolates collected from 2006 to 2012 were previously pathotyped using a distinct differential set of plants (Costamilan et al., 2013). Data analyses were performed in the R software, using the *hagis* package to estimate the distribution of

susceptible reactions, pathotype complexity, pathotype frequency and diversity indices (McCoy et al 2019). Furthermore, the pathotypes were identified using an octal code, which is characterized by assessing the susceptibility responses of the *Rps* gene.

2.4. Microsatellite Genotyping

Total DNA of the *P. sojae isolates* was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The genomic DNA was appropriately diluted to achieve a final working concentration of 10ng/µL. This DNA served as the template for a PCR multiplex designed to amplify the 21 SSR markers (Supplementary Table 1).

Forward primers were labeled with the fluorophores 6-FAM, VIC, NED, or PET. The PCR reaction was performed with the 1X Type-it Microsatellite PCR Master Mix (Qiagen, Hilden, Germany) following the manufacturer's protocol (New England Biolabs, Inc.) arranged in a multiplex PCR (Supplementary Table 2). Multiplex PCR cycles were performed on a T100 thermocycler (Bio-rad, California, USA) and consisted of an initial denaturation step of 5 min at 95°C, followed by 30 cycles of 95°C for 30 s, 58°C for 180 s and 72°C for 30 s and a final elongation step of 30 min at 60°C. The PCR product was sent to Macrogen Inc. for analysis with the GeneScan - 500 LIZ standard size (Applied Biosystems) on an ABI 3730xl sequencer. The fragments were analyzed using GENEMARKER v.1.191 software (SoftGenetics). The size of DNA fragments was manually binned into alleles based on the number of repeat units at each locus.

2.5. Data Analysis

The poppr R package version 2.9.3 (Kamvar et al. 2014) was used to calculate gene diversity (Nei 1978) by locus, the number of multilocus genotypes (MLGs), and the index of association (IA) and the standardized index of association (rbarD). Diversity indices such as genotype richness, the exponential of Shannon's entropy, and the inverse of Simpson's concentration were estimated using Hill numbers (N) of orders 0, 1, and 2, respectively, for the entire population (Chao et al. 2014; Chao et al. 2014). The iNEXT package (version 2.0.20) was employed to assess interpolation (rarefaction) and extrapolation curves of the sampled population for comparing the three diversity orders of the Hill number (Hsieh et al. 2016). Analyses were conducted with clone correction using the clonecorrect function from the poppr R package for the overall population. A principal components analysis (PCA) was performed on the dataset without any population assignment to identify clusters of individuals. The adegenet R package (Jombart 2008; Jombart and Ahmed 2011) was utilized to create a minimum spanning network based on Bruvo's genetic distance (Kamvar et al. 2014; 2015).

3. Results

3.1. Isolates of *P. sojae*

All 119 isolates were characterized for their virulence phenotypes (pathotypes) using a differential-host set and genotyped with SSR markers. These isolates were collected from seven Brazilian states: Rio Grande do Sul (RS), Paraná (PR), Minas Gerais (MG), Mato Grosso do Sul (MS), Santa Catarina (SC), Goiás (GO), and São Paulo (SP) (Figure 1). additional information for each isolate is available in Supplementary Table 3.

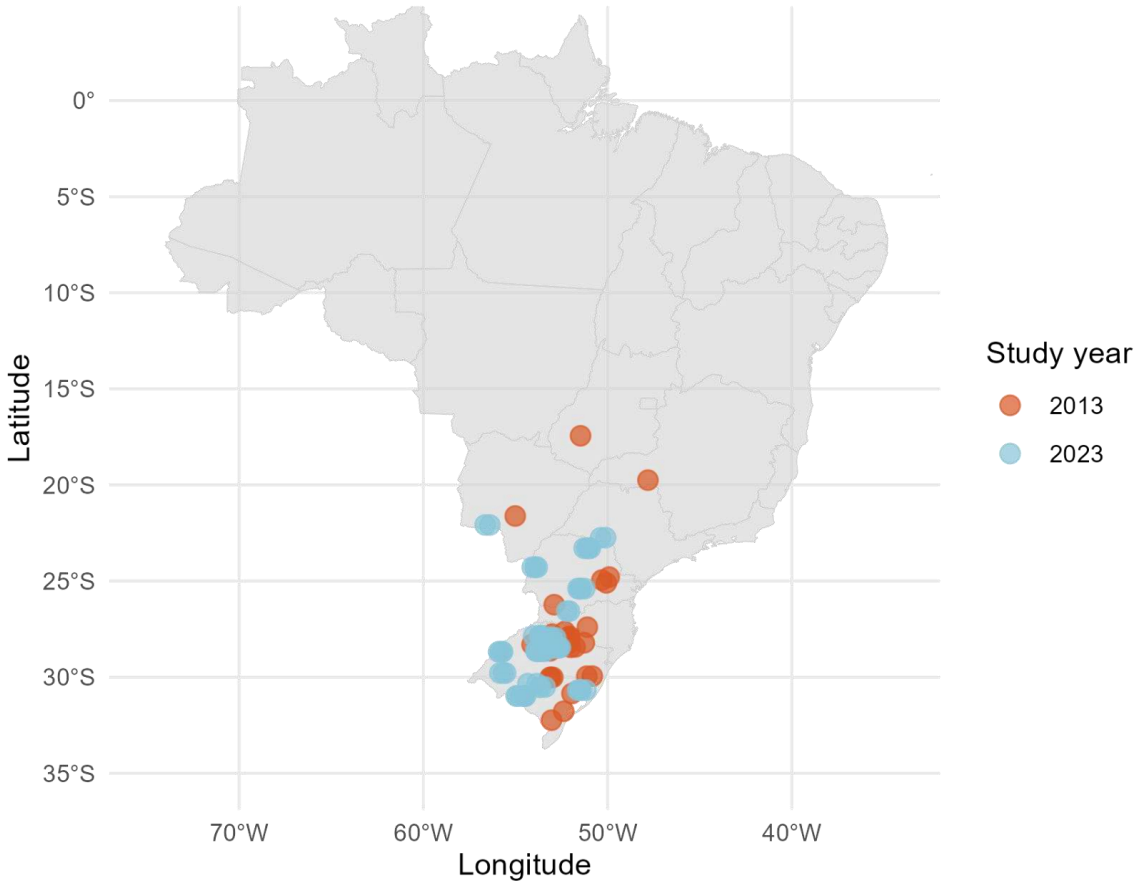

Figure 1. Geographical map illustrating the distribution of isolates of *Phytophthora sojae* collected during the 2013 and 2023 studies.

3.2. Pathotypes of *P. sojae*

The study investigated isolates from two distinct periods, 2013 and 2023. In 2013, 37 samples were analyzed, revealing the presence of 17 unique pathotypes with a mean complexity of 6.70. Comparatively, in the 2023 study, which involved a larger sample size of 74, 37 unique pathotypes were identified with a higher mean complexity of 9.74 (Table 1, Supplementary Figure 3).

The prevalence of virulent isolates affecting the *Rps* gene/allele in each study period revealed variation of virulence between the two samplings. More significant changes were observed concerning allelic variation in *Rps*1a, *Rps*1b, *Rps*1c, and *Rps*1k when comparing populations from both studies. The percentage of isolates

capable of overcoming these genes in 2013 was less than 12%. In contrast, in the 2023 study, this percentage increased to 20% for *Rps*1a and *Rps*1c, up to 40% for *Rps*1k and up to 80% *Rps*1b (Supplementary Figure 1).

Table 1. Number of pathotypes, mean pathotype complexity and diversity indices of *Phytophthora sojae* isolates from different studies in Brazil.

Pop	N	Pathotypes	Simple index	Gleason	Shannon	Simpson	Evenness	Complexity mean
2013	37	17	0.45	4.43	2.53	0.89	0.89	6.70
2023	74	36	0.48	8.13	3.17	0.93	0.88	9.74
Total	119	53	-	-	-	-	-	-

3.3. Genotypic and gene diversity

Twenty-one SSR primers (loci) were initially used to investigate the genetic structure of *P. sojae* in Brazil. However, two loci were excluded: one did not produce detectable peaks, and the other due to uncertainty when binning. All remaining 19 loci were polymorphic. The number of alleles per locus varied from 2 to 14 and the total number of alleles for the 14 loci was 131 (Table 2). The genotype accumulation curve showed a plateau, indicating that the SSR markers have enough power to describe a significant number of MLGs (Supplementary Figure 4).

Among the 119 *P. sojae* isolates, 96 distinct MLGs were detected. Genotype diversity was high for all sampling periods, 2013 and 2023. The number of MLGs for 2013, 2018 and 2023 datasets were 36, 9, and 52, respectively (Table 3). The clonal fraction varied from 0 to 0.28 (Table 3).

Table 2. Allelic diversity metrics estimated using SSR makers for the isolates of *P. sojae* collected in in Brazil.

Locus	Allele	1-D	Hexp
PS01	12.00	0.75	0.76
PS04	10.00	0.75	0.75
PS05	14.00	0.83	0.83
PS07	7.00	0.61	0.61
PS16	7.00	0.77	0.77
PS06	5.00	0.51	0.51
PS10	11.00	0.84	0.84
PS30	6.00	0.68	0.69
PS24	3.00	0.43	0.43
PS18	5.00	0.68	0.68
PS12	8.00	0.76	0.77
PS19	5.00	0.43	0.43
PS25	6.00	0.62	0.62
PS29	2.00	0.31	0.31
PS20	9.00	0.71	0.72
PS36	3.00	0.50	0.51
PS38	5.00	0.59	0.60
PS37	7.00	0.61	0.61
PS27	6.00	0.63	0.63
mean (total)	6.89 (131)	0.63	0.63

Allele = Number of observed alleles; 1-D = Simpson index; Hexp = Nei's 1978 gene diversity

Table 3. Genotypic and genetic diversity of *P. sojae* isolates from populations across multiple study years in Brazil.

Pop	N	MLG	Clonal fraction	H	G	lambda	E.5	Hexp	Ia	rbarD
2013	37	36	0.02	3.57	35.1	0.972	0.985	0.427	6.464	0.3629
2018	9	9	0	2.20	9.0	0.889	1.000	0.652	0.878	0.0515
2023	73	52	0.28	3.77	33.1	0.970	0.757	0.562	2.213	0.1237
total	119	96	0.81	4.44	67.1	0.985	0.792	0.635	3.571	0.2002

MLG = Number of multilocus microsatellite genotypes; H = Shannon-Wiener Index; G = Stoddart and Taylor's Index; lambda = Simpson's Index; E.5 = Evenness; Hexp = Nei's diversity; Ia = Index of association; rbarD = Standardized index of association.

3.4. Population differentiation

3.4.1. AMOVA

AMOVA was performed to assess the partition of genetic variation according to crop season and state of origin across multiple study years. There was some genetic structuring. The percent of variation among seasons was 19.59 % and 25.19 % among studies (Table 4). A significant portion of the genetic variance was observed between samples within the same state at both the season-by-state (66.88%) and study-by-state (63.02%) hierarchical levels (Table 4). At the season-by-state hierarchical level, genetic variation between the different crops represented 19.59% of the total variation. The Φ statistic of 0.19 indicates a moderate genetic correlation between growing seasons, suggesting that while there is observable genetic differentiation between seasons, it is not markedly pronounced. Similarly, at the study-by-state hierarchical level the genetic variation was 25.19% within different studies conducted within the

same state. The Φ statistic was 0.25. Thus, taken together, there is moderate genetic correlation between various studies.

Table 4. Analysis of molecular variance table generated comparing *Phytophthora sojae* isolates for two different hierarchies.

Hierarchy	df	Sum of squares (Mean)	Variation (%)	Φ statistic
Season by state				
Between Season	8	586 (73.33)	19.59	0.19
Between State Within Season	11	226 (20.56)	2.66	0.03
Between samples Within State	80	1481 (18.52)	66.88	0.86
Within samples	100	139 (1.39)	10.85	0.89
Total	199	2433 (12.23)	100	
Study by state				
Between Study	2	441 (220)	25.19	0.25
Between State Within Study	11	206 (20.60)	1.67	0.02
Between samples Within State	80	1600 (18.83)	63.02	0.86
Within samples	100	137 (1.39)	10.10	0.89
Total	199	2384 (12.22)	100	

df = degrees of freedom.

3.4.2. PCA and DAPC

The PCA did not reveal a clear differentiation among populations across different strata, study years, states, or seasons (Supplementary Figure 5). Only one component accounted for 31.7% of the variance, while the second component

explained 18.6%. These results suggest a lack of distinct separation or patterns among the studied populations, irrespective of various strata or temporal factors.

The discriminant analysis of principal components (DAPC) was run using the first discriminant component and isolates could be separated across multiple study years into three clusters with assignment probability of 99.2% (Figure 2).

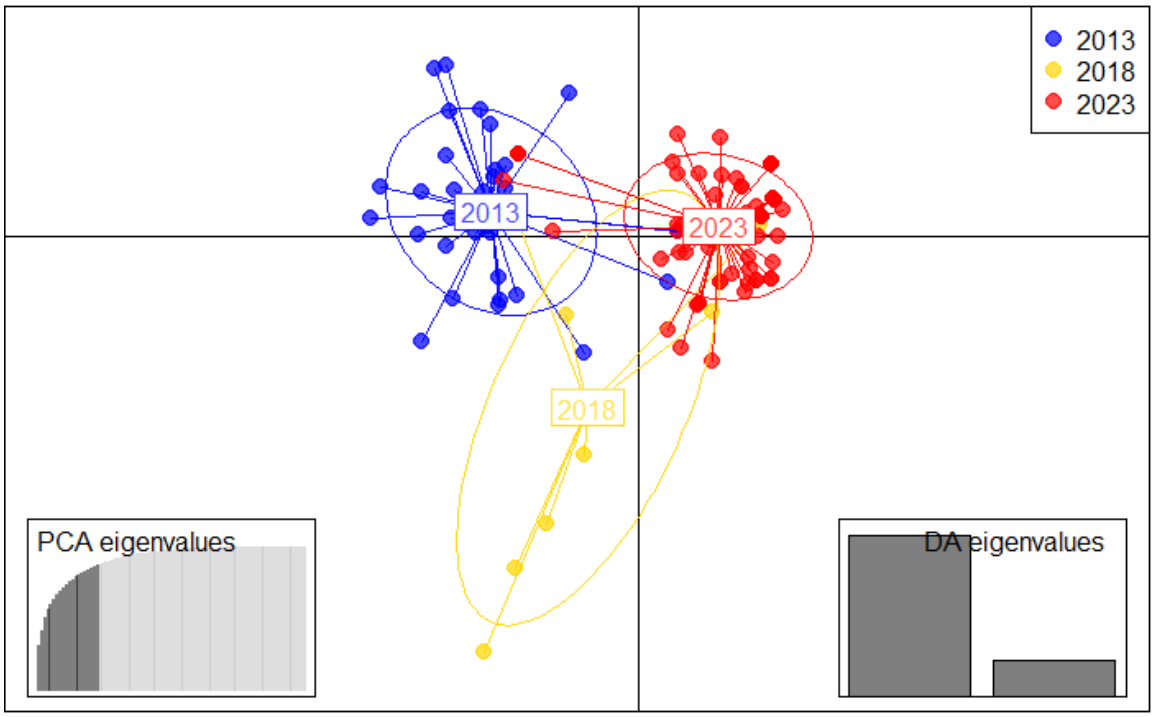

Figure 2. Discriminant analysis of principal components (DAPC)

3.4.3. Minimum spanning network

A minimum spanning network (MSN) showed that the *P. sojae* population in the 2023 study had a greater number of MLGs compared to the 2013 population, it also indicates that clustering in the study aligns with the respective time periods (Figure 3). Overall, individuals from the 2013 population tend to cluster among themselves, while those from the 2023 population primarily group together among individuals from the same year. This trend was observed in other networks created to better visualize the relationship between genotypes and different stratifications (Supplementary Figure 6).

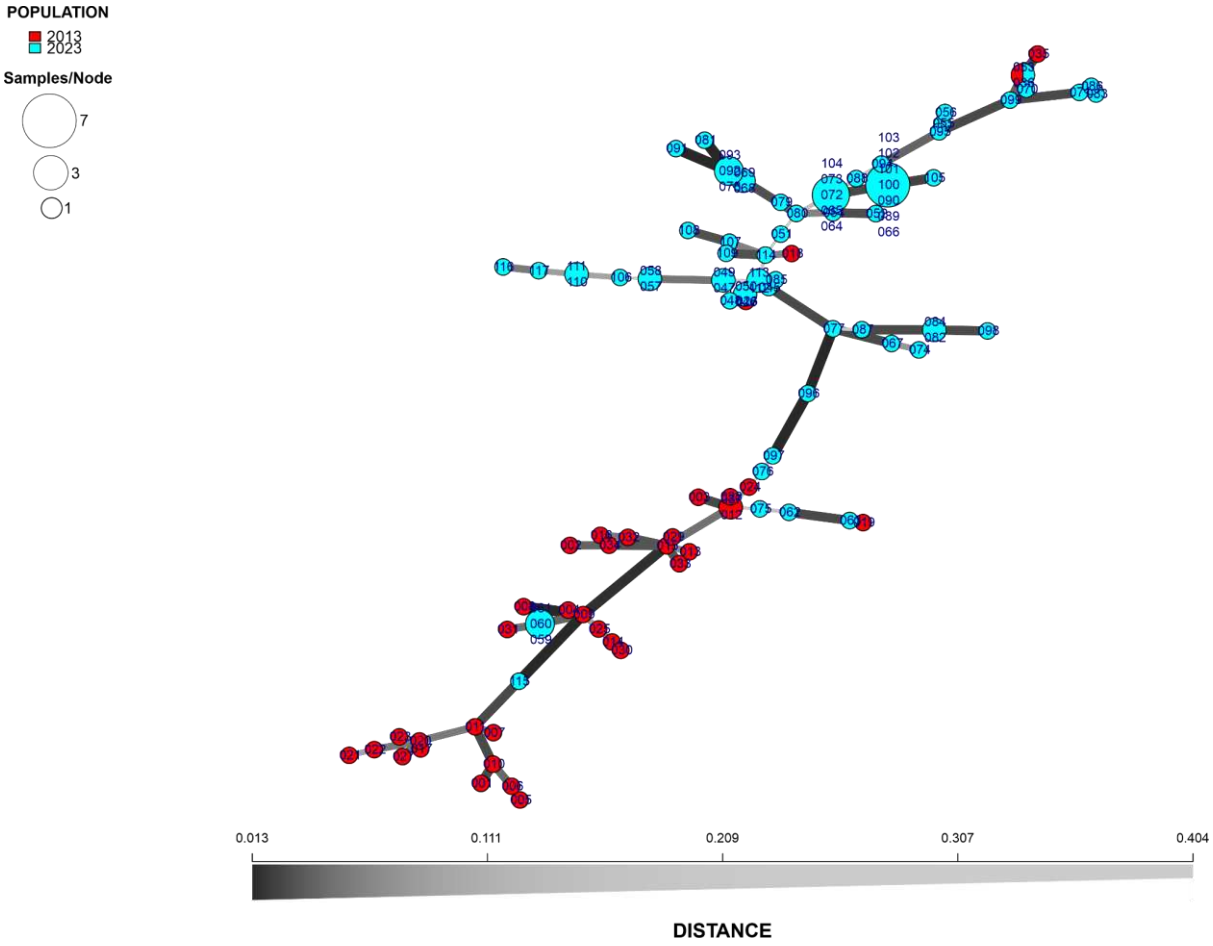

Figure 3. Minimum Spanning Network (MSN) illustrating the genetic relationships among *P. sojae* populations from 2013 and 2023 studies.

4. Discussion

Given the prevalence of the SRSR disease caused by *P. sojae*, this study aims to deepen the understanding of the evolutionary dynamics of this pathogen in Brazilian soybean producing areas. This study seeks to analyze the genetic variability and temporal dynamics of the *P. sojae* population, with a focus on genetic and pathotype diversity, in order to direct more effective and sustainable management strategies of SRSR in Brazil, the main global producer of this important agricultural crop.

The investigation of *P. sojae* pathotypes in different periods revealed significant changes in the complexity of the pathotypes over the years (Supplementary Figure 3). Although the dataset for the 2023 population was larger than the dataset used in the

previous comparative study (Batista et al., 2023), the current investigation did not reveal significant changes in pathotype diversity. The diversity remained consistently high (Supplementary Figure 2).

The change in diversity and complexity of *P. sojae* pathotypes in Brazil over the years was similar to that reported in a comparative global-temporal analysis study on the efficacy of *P. sojae Rps* genes. This study also identified temporal changes in the complexity and diversity of *P. sojae* pathotypes in countries such as the United States and Argentina, which are among the main soybean producers (McCoy et al., 2023). Additionally, the efficiency of *Rps* genes incorporated in commercial cultivars across populations in the United States, Argentina, China, and Canada was highlighted. Similarly, Batista et al. (2023) reported comparable findings for these genes when analyzing the *P. sojae* population in Brazil. *Rps* genes 1a, 1c, and 1k are not very effective in the current *P. sojae* populations in both countries.

The shift in complexity of *P. sojae* has been an object of investigation in previous *P. sojae* populations studies. Dorrance and Grünwald (2008) linked this alteration in the Australian population to genetic mutations. In contrast, as suggested by Costamilan et al. (2013), the shift is hypothesized to result from selection pressure due to the wide-scale utilization of cultivars containing the *Rps* gene and the extensive incorporation of soil conservation methods. Stewart et al. (2014) proposed an alternative hypothesis for the high genetic diversity, suggesting the potential occurrence of outcrossing between *P. sojae* isolates during co-infection in the field, since such events were easily induced under laboratory conditions.

The increasing diversity of pathotypes could challenge the effectiveness of existing resistance strategies. This may necessitate the adaptation or development of new control strategies to preserve soybean plant resistance. Understanding the

evolutionary dynamics of the pathogen is crucial in guiding future agricultural practices and the development of more resilient soybean varieties. To achieve this, we used SSR markers for genotyping the isolates. These markers had enough resolution to improve the estimation of the amount of genetic variation and the virulence characteristics of *P. sojae* isolates. SSR markers have been proven instrumental in investigating evolutionary history, population structures, and migration patterns within populations (Kerio et al. 2019).

The genotypic analysis revealed a pattern of continuity in the genetic variability of *P. sojae* between the 2013 and 2023 studies. Contrary to the expectation of contrasting changes, both samples presented similar levels of genetic diversity. In addition, the data analysis did not reveal any prevalence of specific genotypes (MLG) at relatively high frequencies. This suggests an absence of persistent dominant lineages. It is important to emphasize that the clonal fraction of the 2013 population stands at 0.02, indicating a near 100% diversity. In comparison, the 2023 population exhibits higher clonal fraction (0.28), although it remains relatively low. There were no major differences in the magnitude of the diversity indices (Shannon-Wiener, Stoddart and Taylor's, Simpson's, and Nei's diversity) when data from the different studies were considered over time.

There was no evidence to support the hypotheses that were formulated upfront. The observation of genetic variation between different populations from surveys carried out in 2013, 2018 and 2023 suggests consistency over time in the genetic structure of the *P. sojae* population. This may indicate stability in the genetic structure of this population, which is inconsistent with the hypothesis of significant variation over time. The genetic structure found between different crops seasons and states of origin also does not support this hypothesis. Similar results were also observed in the

population of Argentina of *P. sojae*, where no signs of genetic subdivision were evidenced at geographic scales (Grijalba et al. 2020).

In light of these findings, it is plausible that selection exerted by the genetic diversity of host cultivars plays a pivotal role in shaping pathogen populations. This contributes not only to the wide variability of pathotypes, but also to the diversity of multilocus genotypes observed. However, information regarding the specific resistance genes present in the commercial soybean varieties cultivated in these regions remains unavailable, as well as the predominant cultivar used for the management of SRSR.

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The authors declare that they have no conflict of interest.

5. References

Batista, I. C. A., Silva, M. P. C., Silva Junior, A. L., Arrigada, M. P. G., de Camargo, M. P., Figueiredo, A., et al. 2023. A shift in pathotype diversity and complexity of *Phytophthora sojae* in Brazil. Plant Dis. 107:1968–1972.

Burdon, J. J., Zhan, J., Barrett, L. G., Papa¨ıx, P., and Thrall, P. H. 2016. Addressing the challenges of pathogen evolution on the world's arable crops. Phytopathol. 106, 1117-1127.

Chao, A., Chiu, C. H., and Jost, L. 2014. Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through hill numbers. Annu. Rev. Ecol. Evol. Syst. 45:297–324.

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., et al. 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecol. Monogr. 84:45–67.

Costamilan, L. M., Clebsch, C. C., Soares, R. M., Seixas, C. D. S., Godoy, C. V., and Dorrance, A. E. 2013. Pathogenic diversity of *Phytophthora sojae* pathotypes from Brazil. Eur. J. Plant Pathol. 135:845–853.

Dorrance, A., and Grünwald, N. J. 2008. *Phytophthora sojae*: Diversity among and within populations. oomycete genetics and genomics: diversity, interactions, and research tools. :197–212.

Dorrance, A. E. 2018. Management of *Phytophthora sojae* of soybean: a review and future perspectives. Can. J. Plant Pathol. 40:210–219.

Faostat. 2023. Food and Agriculture Organization of the United Nations. Available at: Available at: http://faostat.fao.org.

Förster, H., Tyler, B. and Coffey, M. 1994. *Phytophthora sojae* races have arisen by clonal evolution and by rare outcrosses. Mol. Plant Microbe Interact. 6, 780–791.

Gijzen M., Qutob D. 2009. Phytophthora sojae and soybean. In: Lamour K., Kamoun S. (eds) Oomycetes genetics and genomics: diversity, interactions, and research tools. Wiley, Hoboken.

Grijalba, P. E., Martínez, M. C., and Guillin, E. 2020. Pathotype and SSR variation in *Phytophthora sojae* from the Argentinean Pampas. J. Phytopathol. 168:228–243.

Hebb, L. M., Bradley, C. A., Mideros, S. X., Telenko, D. E. P., Wise, K., and Dorrance, A. E. 2022. Pathotype complexity and genetic characterization of *Phytophthora sojae* populations in Illinois, Indiana, Kentucky, and Ohio. Phytopathology. 112:663–681.

Jombart, T., and Bateman, A. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics. 24:1403–1405.

Jombart, T., and Ahmed, I. 2011. Adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. Bioinformatics 27:3070-3071.

Kamvar, Z. N., Tabima, J. F., and Grünwald, N. J. 2014. *Poppr*: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ. 2:e281.

Kamvar, Z. N., Brooks, J. C., and Grünwald, N. J. 2015. Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. Front. Genet. 6:208.

Lin, F., Chhapekar, S. S., Vieira, C. C., Da Silva, M. P., Rojas, A., Lee, D., et al. 2022. Breeding for disease resistance in soybean: a global perspective. Theor. Appl. Genet. 1–100.

Matthiesen, R. L., Schmidt, C., and Robertson, A. E. 2021. Comparison of baiting techniques for recovering *Phytophthora sojae* from soybean fields in Iowa. Plant Heal. Prog. 22:316– 322.

McCoy, A. G., Noel, Z., Sparks, A. H., and Chilvers, M. 2019. hagis, an R Package Resource for Pathotype Analysis of Phytophthora sojae Populations Causing Stem and Root Rot of Soybean. Mol. Plant-Microbe Interact. 32:1574–1576.

McCoy, A. G., Belanger, R. R., Bradley, C. A., Cerritos-Garcia, D. G., Garnica, V. C., Giesler, L. J., et al. 2023. A global-temporal analysis on *Phytophthora sojae* resistance-gene efficacy. Nat. Commun. 14:6043.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Stewart, S., Abeysekara, N., and Robertson, A. E. 2014. Pathotype and genetic shifts in a population of *Phytophthora sojae* under soybean cultivar rotation. Plant Disease, 98(5), 614-624.

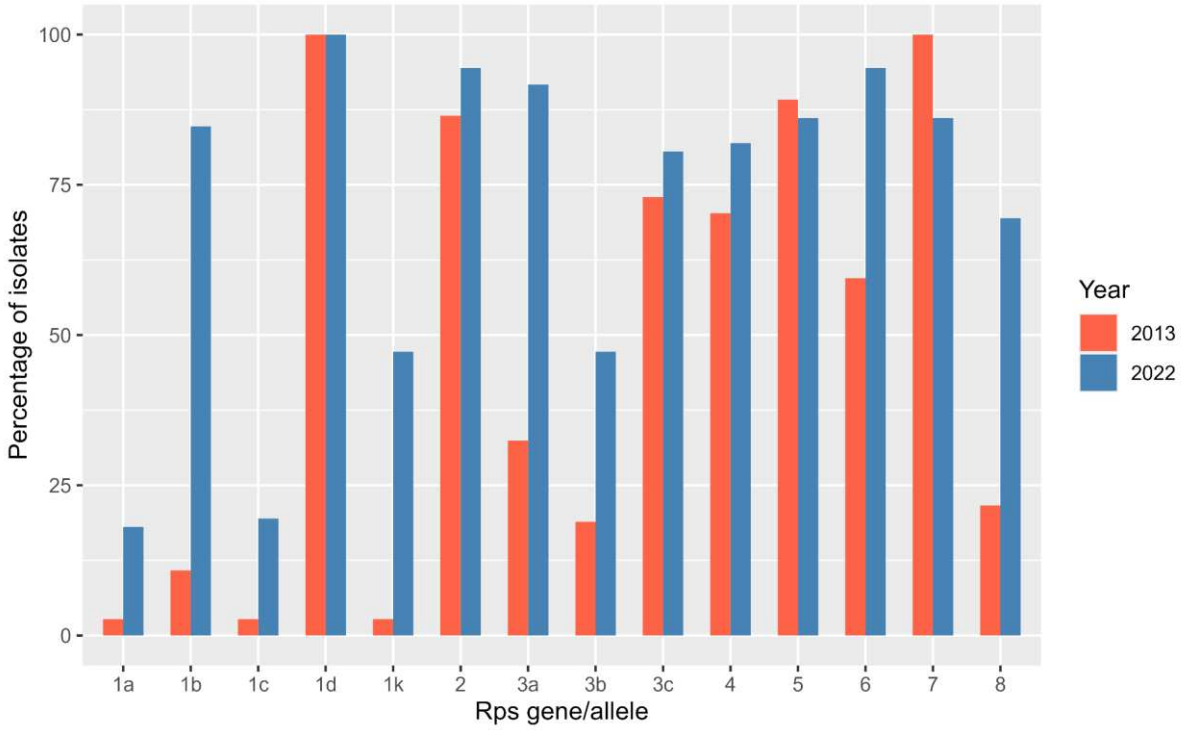
Stewart, S., Robertson, A. E., Wickramasinghe, D., Draper, M. A., Michel, A., and Dorrance, A. E. 2016. Population structure among and within Iowa, Missouri, Ohio, and South Dakota populations of *Phytophthora sojae*. Plant Dis. 100:367–379.

Zdziarski, A. D., Todeschini, M. H., Milioli, A. S., Woyann, L. G., Madureira, A., Stoco, M. G., et al. 2018. Key soybean maturity groups to increase grain yield in Brazil. Crop Sci. 58.

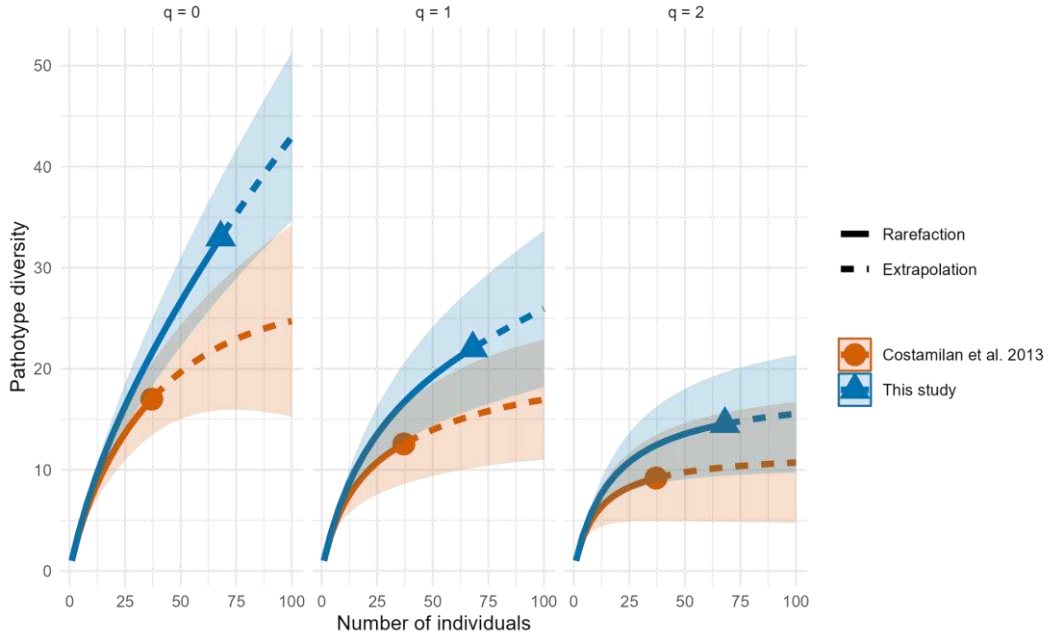
Supplementary data

CHAPTER 3 - Mapping pathotype variability and genetics of *Phytophthora sojae* in Brazil

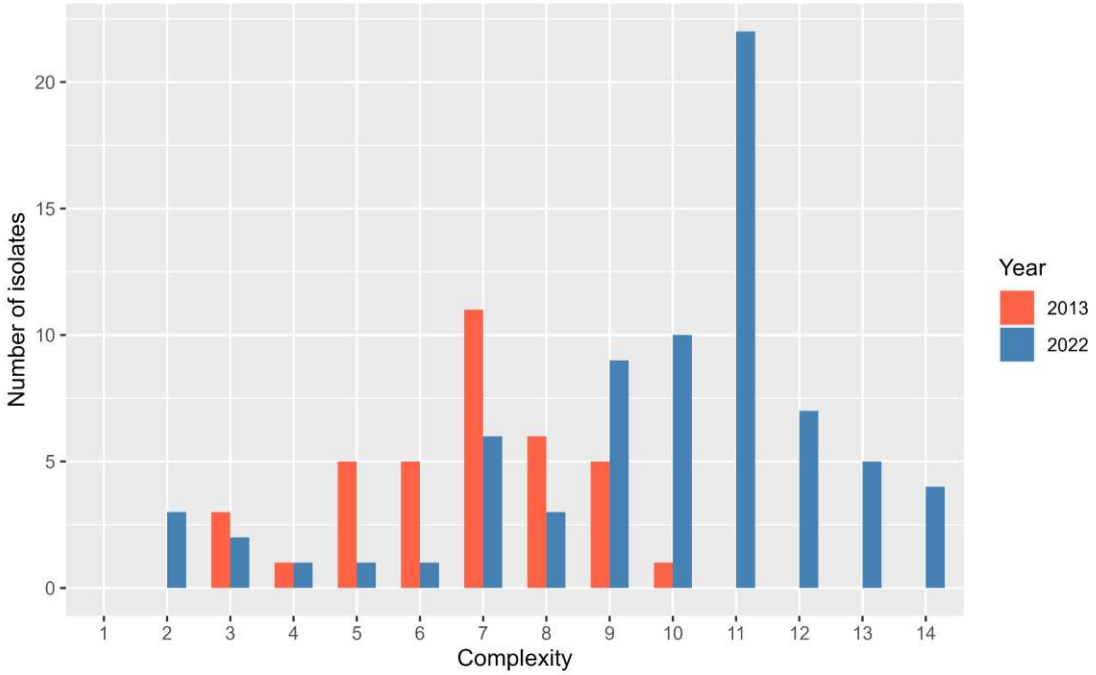
I. C. A. Batista, L. M. Costamilan, A. L. Silva Junior, A. F. Silva, G. C. F. de Melo, and E. S. G. Mizubuti



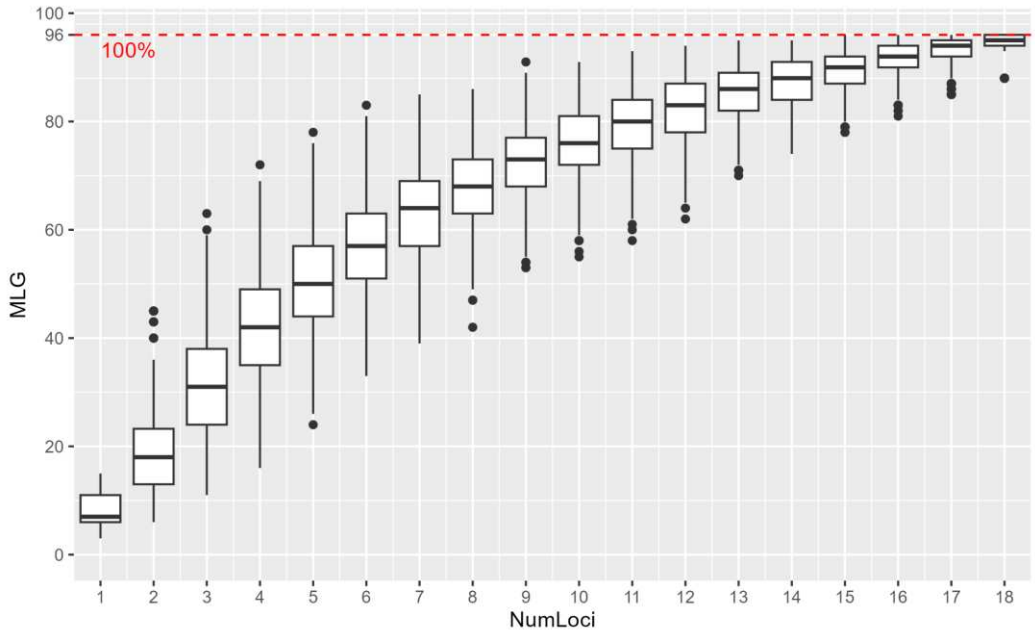
Supplementary Figure 1. Percentage of *Phytophthora sojae* isolates recovered in a survey conducted in soybean fields in both 2013 and 2023 studies, displaying their virulence on a specific Rps gene/allele.



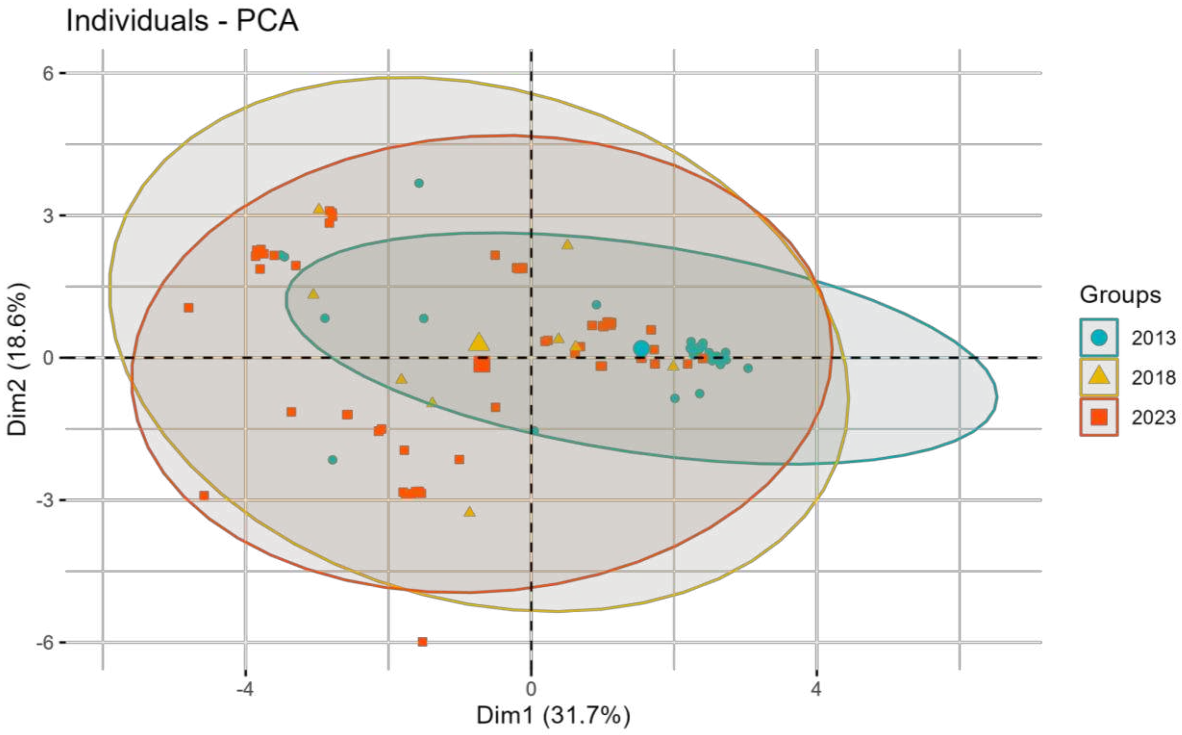
Supplementary Figure 2. Rarefaction curves based on Hill numbers of orders q = 0 (richness), 1 (exponential of Shannon's entropy index) and 2 (inverse of Simpson's concentration index) used to estimate diversity of pathotypes of *Phytophthora sojae* from the study by Costamilan et al. (2013) (N = 37) and from this study (2020/21; N = 74). Interpolation (solid), extrapolation (dashed) lines and their 95% confidence intervals (shaded area) are indicated.



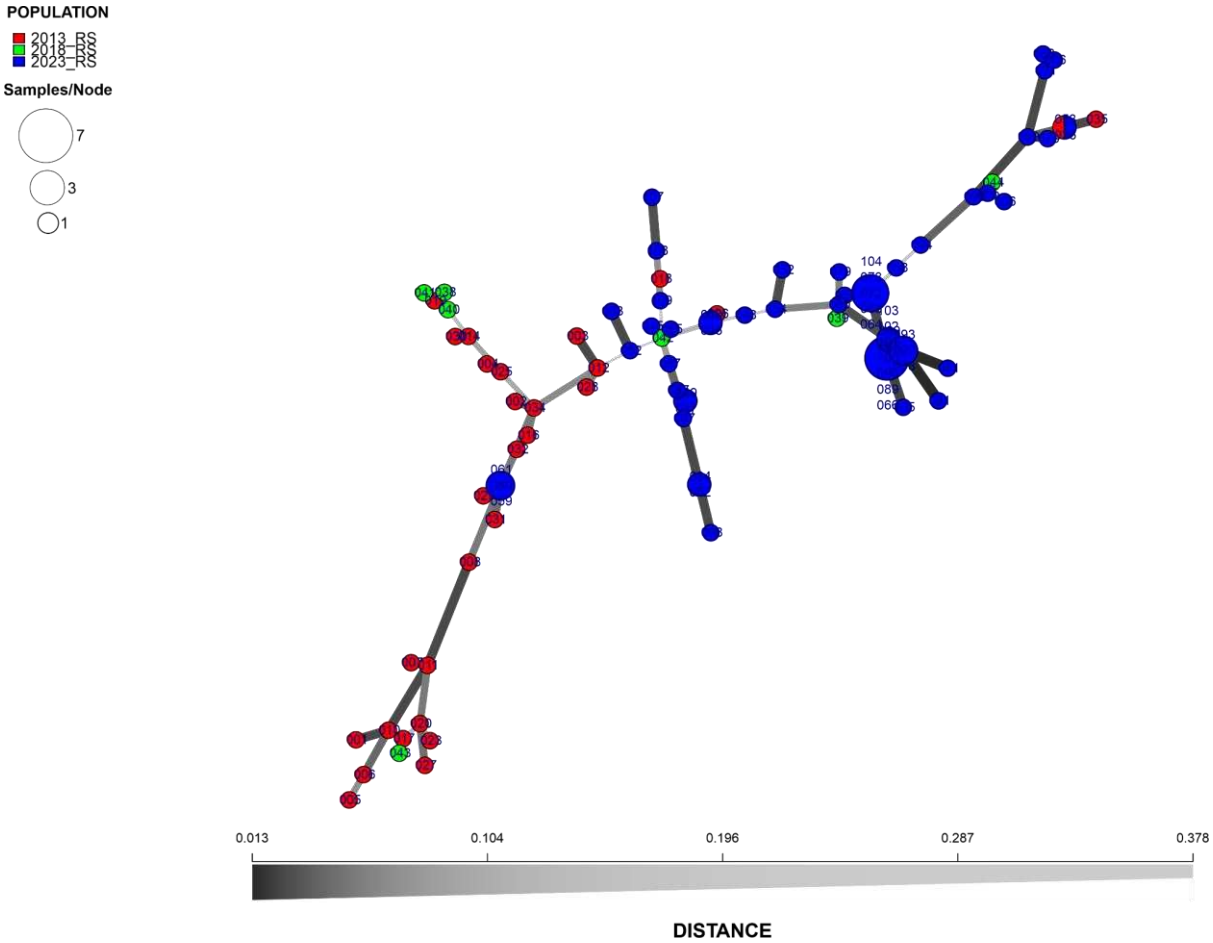
Supplementary Figure 3. Number of isolates of *Phytophthora sojae* that were capable of overcoming different *Rps* genes (Complexity). Isolates obtained from the South region of Brazil in two periods of time.



Supplementary Figure 4. Genotype accumulation curve for *Phytophthora sojae* isolates. Each boxplot contains 1,000 random samples representing different possible combinations of n loci. The horizontal red dashed line represents 100% of MLG resolution.



Supplementary Figure 5. Scatter plot of principal component analysis (PCA) for the genotypes of *Phytophthora sojae* isolates collected in Brazil.



Supplementary Figure 6. Minimum Spanning Network displaying genetic relationships among *P. sojae* isolates collected exclusively within Rio Grande do Sul, across different study years (2013, 2018 and 2023).

Supplementary Table 1. Primer sets utilized for genotyping analysis.

Primer		sequence (3'-5')	Dye labbel
PS01	Fwd	TGATGGGAGATGGCTACAGG	6FAM
	Rev	TCGCAACGACAGATTGATG	-
PS04	Fwd	CTTCCCATCACTCCGACAAG	NED
	Rev	TTGACACTGCCTCCTACACG	-
PS05	Fwd	GAAACAATCAACCGAACAACG	VIC
	Rev	ATAGGAGGGCAAACTGGATG	-
PS06	Fwd	AACTACCTTCGCTCGTGGTG	NED
	Rev	CCTATCGCCTGGAAAATGTG	-
PS07	Fwd	TCCTTAGCTTCCGGTTAAGC	PET
	Rev	TCTCATTTTGGCCTGGAAAC	-
PS10	Fwd	CGACGAAGAACAACATTACTTG	VIC
	Rev	ATGAAACCGAACCAAACCTG	-
PS12	Fwd	GCTGCTTGTTGCTGTTGTTG	VIC
	Rev	GCGGGTGTTTGGAGAGTATC	-
PS16	Fwd	AATCTGACTTGGACGCTGTG	6FAM
	Rev	GCTTAGTGTTTTGGGTTACGC	-
PS17	Fwd	GAAGCCGAAGACGAAAAGAG	PET
	Rev	TGAAAAAGTGACCAAACAGTGG	-
PS18	Fwd	TCCAGGTTGAGGTGACTTG	NED
	Rev	GAAGAGCGTGAGCAGGAAC	-
PS19	Fwd	CGTGATGGAGCACTCAGAAG	PET
	Rev	CGCAATCTTCCTGCTAATGG	-
PS20	Fwd	AAATCCAACCAGCCTTACCC	PET
	Rev	CGTGCTTCATGTCTGCACTAC	-
PS24	Fwd	GTCATTTCCCTCGCTCACAC	6FAM
	Rev	ACACTGGCAACAAGCAACAG	-

PS25	Fwd	AGCGTTGGTGTTGACGAC	NED
	Rev	TAGCGAAACCTGGCAAATG	-
PS27	Fwd	TTCAGATTCCGTGAGCATTG	PET
	Rev	CGGCTTGGTCTCTTAGCTTC	-
PS29	Fwd	CCACTGAAGCGAGGTAGAGG	VIC
	Rev	GTAGCACAAAATCCGTCTGC	-
PS30	Fwd	ACGAAGTCCAACCATAAATCG	VIC
	Rev	TGAAAGATGACCCCAGGATG	-
PS33	Fwd	CTGCTAGTGCCGTTCGTTG	6FAM
	Rev	TAAAAGGGCTGCTCAAATCG	-
PS36	Fwd	CAAAAATCATCAGCACCTTCG	6FAM
	Rev	TAGCCAAAAGAGCGACAACC	-
PS37	Fwd	ACGAGCCCACGGAAGAGTTC	VIC
	Rev	CTGGATGATGTGCGGGTTTG	-
PS38	Fwd	AGTGGGCGTTTGCTTGTG	NED
	Rev	TCGCTGTGGTTCCTCTTCTC	-

Supplementary Table 2. Schematic representation of the SSR multiplex PCR for genotyping.

Multiplex01		Multiplex02		Multiplex03		Multiplex04		Multiplex05		Multiplex06	
PS01	**200-245**	**PS16**	**400-500**	**PS24**	**220-260**	**PS33**	**240-267**	PS36	150-220	PS17	180-230
PS04	250-320	PS06	160-200	PS18	170-200	PS25	350-400	PS38	245-270	PS30	270-300
PS05	**200-300**	**PS10**	**150-250**	**PS12**	**220-306**	**PS29**	**250-280**	PS37	200-370		
PS07	220-240			PS19	225-275	PS20	150-225	PS27	275-325		

Colors represent the fluorescent dye set: blue = ¨6FAM; yellow = NED; green = VIC; red = PET.

Supplementary Table 3. Isolates of *Phytophthora sojae* from different soybean-producing areas in the South region of Brazil.

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
EPs01	Plant	2013	2006/07	NA	Passo Fundo	RS	1d, 2, 3b, 3c, 4, 5, 6, 7	8	5671	MLG 90	Costamilan et al. 2013
EPs02	Plant	2013	2006/07	NA	Passo Fundo	RS	1d, 2, 3b, 3c, 4, 5, 6, 7	8	5671	MLG 73	Costamilan et al. 2013
EPs03	Plant	2013	2006/07	NA	Passo Fundo	RS	1d, 2, 3b, 3c, 4, 5, 6, 7	8	5671	MLG 78	Costamilan et al. 2013
EPs04	Plant	2013	2006/07	NA	Passo Fundo	RS	1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	9	5771	MLG 88	Costamilan et al. 2013

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
EPs05	Plant	2013	2006/07	NA	Passo Fundo	RS	1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	9	5771	MLG 62	Costamilan et al. 2013
EPs06	Plant	2013	2006/07	NA	Passo Fundo	RS	1d, 2, 3b, 3c, 4, 5, 6, 7	8	5671	MLG 64	Costamilan et al. 2013
EPs07	Plant	2013	2006/07	NA	Coxilha	RS	1d, 2, 3c, 4, 5, 6, 7	7	5471	MLG 47	Costamilan et al. 2013
EPs08	Plant	2013	2006/07	NA	Coxilha	RS	1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	9	5771	MLG 43	Costamilan et al. 2013
EPs09	Plant	2013	2006/07	NA	Ponta Grossa	PR	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	10	25771	MLG 81	Costamilan et al. 2013
EPs10	Plant	2013	2007/08	NA	Ronda Alta	RS	1b, 1d, 2, 3b, 3c, 4, 5, 6, 7	9	25671	MLG 91	Costamilan et al. 2013
EPs11	Plant	2013	2007/08	NA	Coxilha	RS	1d, 2, 3b, 3c, 4, 5, 6, 7	8	5671	MLG 86	Costamilan et al. 2013
EPs12	Plant	2013	2007/08	NA	Coxilha	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	10	25771	MLG 80	Costamilan et al. 2013
EPs13	Plant	2013	2007/08	NA	Uberaba	MG	1b, 1d, 2, 3b, 3c, 4, 5, 6, 7	9	25671	MLG 71	Costamilan et al. 2013
EPs14	Plant	2013	2007/08	NA	Cachoeirinha	RS	1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	10	5773	MLG 87	Costamilan et al. 2013
EPs15	Plant	2013	2007/08	NA	Carambeí	PR	1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	10	5773	MLG 96	Costamilan et al. 2013
EPs16	Plant	2013	2007/08	NA	Santo Ângelo	RS	1d, 2, 3b, 3c, 4, 5, 6, 7	8	5671	MLG 76	Costamilan et al. 2013
EPs17	Plant	2013	2007/08	NA	Passo Fundo	RS	1d, 3a, 5, 7, 8	5	1123	MLG 30	Costamilan et al. 2013

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
EPs18	Plant	2013	2007/08	NA	Pelotas	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 27	Costamilan et al. 2013
EPs19	Plant	2013	2007/08	NA	Arroio Grande	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 45	Costamilan et al. 2013
EPs20	Plant	2013	2007/08	NA	Camaquã	RS	1d, 2, 3a, 3b, 4, 5, 6, 7, 8	9	5373	MLG 85	Costamilan et al. 2013
EPs21	Plant	2013	2007/08	NA	Maracaju	MS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	10	25773	MLG 41	Costamilan et al. 2013
EPs22	Plant	2013	2009/10	NA	Pato Branco	PR	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	10	25771	MLG 61	Costamilan et al. 2013
EPs23	Plant	2013	2009/10	NA	Colorado	RS	1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	9	5771	MLG 63	Costamilan et al. 2013
EPs24	Plant	2013	2009/10	NA	Castro	PR	1d, 2, 3b, 3c, 4, 5, 6, 7	8	5671	MLG 75	Costamilan et al. 2013
EPs25	Plant	2013	2009/10	NA	Ipiranga do Sul	RS	1b, 1d, 2, 3b, 3c, 4, 5, 6, 7	9	25671	MLG 89	Costamilan et al. 2013
EPs26	Plant	2013	2009/10	NA	Cachoeirinha	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 25	Costamilan et al. 2013
EPs27	Plant	2013	2009/10	NA	Chapada	RS	1d, 2, 3a, 3b, 4, 5, 7, 8	8	5373	MLG 84	Costamilan et al. 2013
EPs28	Plant	2013	2009/10	NA	Sananduva	RS	1d, 2, 3a, 5, 7, 8	6	5123	MLG 98	Costamilan et al. 2013
EPs29	Plant	2013	2009/10	NA	Marau	RS	1b, 1d, 2, 3a, 4, 5, 6, 7, 8	9	25373	MLG 83	Costamilan et al. 2013

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
EPs30	Plant	2013	2009/10	NA	Não-Me-Toque	RS	1d, 2, 3a, 3b, 4, 5, 7, 8	8	5773	MLG 42	Costamilan et al. 2013
EPs31	Plant	2013	2009/10	NA	Ijuí	RS	1d, 2, 3b, 3c, 4, 5, 6, 7	8	5671	MLG 70	Costamilan et al. 2013
EPs32	Plant	2013	2009/10	NA	Lagoa Vermelha	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	10	25771	MLG 79	Costamilan et al. 2013
EPs33	Plant	2013	2009/10	NA	Campos Novos	SC	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	10	25771	MLG 65	Costamilan et al. 2013
EPs34	Plant	2013	2009/10	NA	Cachoeira do Sul	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 4, 6, 7	11	77771	MLG 77	Costamilan et al. 2013
EPs35	Plant	2013	2009/10	NA	Cachoeira do Sul	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 16	Costamilan et al. 2013
EPs36	Plant	2013	2009/10	NA	Cachoeira do Sul	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	10	25771	MLG 17	Costamilan et al. 2013
EPs37	Plant	2013	2009/10	NA	Montividiu	GO	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	10	25771	MLG 80	Costamilan et al. 2013
EPs39*	Plant	2013	2017/18	NA	Carazinho	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	14	77773	MLG 12	Costamilan 2023 (unpublished)
EPs40*	Plant	2013	2017/18	NA	Getúlio Vargas	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	14	77773	MLG 8	Costamilan 2023 (unpublished)
EPs41*	Plant	2013	2018/19	NA	Passo Fundo	RS	1a, 1c, 1d, 1k, 2, 4, 5, 7, 8	9	57573	MLG 37	Costamilan 2023 (unpublished)

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
EPs42*	Plant	2013	2018/19	NA	Gentil	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	13	77773	MLG 24	Costamilan 2023 (unpublished)
EPs43*	Plant	2013	2018/19	NA	Ibirubá	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	14	77773	MLG 59	Costamilan 2023 (unpublished)
EPs44*	Plant	2013	2018/19	NA	Erechim	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	12	27773	MLG 31	Costamilan 2023 (unpublished)
EPs45	Plant	2013	2018/19	NA	Passo Fundo	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	14	77773	MLG 33	Costamilan 2023 (unpublished)
Ps001	Soil	2023	2020/21	Leaf-bait	Não Me toque	RS	1b, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7	10	27571	MLG 56	Batista et al. 2023
Ps002	Soil	2023	2020/21	Leaf-bait	Não Me toque	RS	1b, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	11	27573	MLG 55	Batista et al. 2023
Ps003	Soil	2023	2020/21	Leaf-bait	Não Me toque	RS	1b, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	11	27573	MLG 32	Batista et al. 2023
Ps004	Soil	2023	2020/21	Leaf-bait	Não Me toque	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 5, 6, 7, 8	11	27763	MLG 40	Batista et al. 2023
Ps005	Soil	2023	2020/21	Leaf-bait	Sarandi	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	12	27773	MLG 32	Batista et al. 2023
Ps006	Soil	2023	2020/21	Leaf-bait	Não Me toque	RS	1b, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	11	27573	MLG 55	Batista et al. 2023
Ps007	Soil	2023	2020/21	Leaf-bait	Tapes	RS	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573	MLG 44	Batista et al. 2023

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
Ps008	Soil	2023	2020/21	Leaf-bait	Sarandi	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	12	27773	MLG 92	Batista et al. 2023
Ps009	Soil	2023	2020/21	Leaf-bait	Tapes	RS	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573	MLG 17	Batista et al. 2023
Ps010	Soil	2023	2020/21	Leaf-bait	Sarandi	RS	1d, 1k, 2, 3a, 4, 5, 6, 7	8	7171	MLG 35	Batista et al. 2023
Ps011	Soil	2023	2020/21	Leaf-bait	Cruz Alta	RS	1c, 1d, 6, 8	4	41042	MLG 3	Batista et al. 2023
Ps012	Soil	2023	2020/21	Leaf-bait	Cruz Alta	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	13	77573	MLG 2	Batista et al. 2023
Ps013	Soil	2023	2020/21	Leaf-bait	Abelardo Luz	SC	1b, 1d, 1k, 2, 3a, 3c, 4, 6, 7	9	27551	MLG 49	Batista et al. 2023
Ps014	Soil	2023	2020/21	Leaf-bait	Abelardo Luz	SC	1b, 1d, 1k, 2, 3c, 5, 6	7	27460	MLG 49	Batista et al. 2023
Ps015	Soil	2023	2020/21	Leaf-bait	Cruz Alta	RS	1d, 2	2	5000	MLG 72	Batista et al. 2023
Ps016	Soil	2023	2020/21	Leaf-bait	Cruz Alta	RS	1d, 2, 4, 5, 6	5	5070	MLG 72	Batista et al. 2023
Ps017	Soil	2023	2020/21	Leaf-bait	Cruz Alta	RS	1d, 4	2	1010	MLG 72	Batista et al. 2023
Ps018	Soil	2023	2020/21	Leaf-bait	Cruz Alta	RS	1a, 5, 6	3	10060	MLG 39	Batista et al. 2023
Ps019	Soil	2023	2020/21	Leaf-bait	Cruz Alta	RS	1d, 4	2	1010	MLG 38	This study
Ps020	Soil	2023	2020/21	Bioassay	Santo Augusto	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	11	27771	MLG 7	Batista et al. 2023

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
Ps021	Soil	2023	2020/21	Bioassay	Santo Augusto	RS	1b, 1d, 1k, 2, 3a, 3b, 6, 7	8	27341	MLG 7	Batista et al. 2023
Ps022	Soil	2023	2020/21	Bioassay	Santo Augusto	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7	11	27771	MLG 6	Batista et al. 2023
Ps023	Soil	2023	2020/21	Bioassay	Santo Augusto	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 6, 7	9	27741	MLG 52	Batista et al. 2023
Ps024	Soil	2023	2020/21	Bioassay	Sarandi	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 6, 7	9	27741	MLG 34	Batista et al. 2023
Ps025	Soil	2023	2020/21	Bioassay	Sarandi	RS	1b, 1d, 1k, 2, 3a, 3b, 4, 5, 6, 7	10	27371	MLG 34	Batista et al. 2023
Ps026	Soil	2023	2020/21	Bioassay	Alegrete	RS	1d, 2, 3a, 3c, 4, 5, 6, 7, 8	9	5573	MLG 22	Batista et al. 2023
Ps027	Soil	2023	2020/21	Bioassay	Alegrete	RS	1b, 1d, 2, 3a, 7, 8	6	25103	MLG 15	Batista et al. 2023
Ps028	Soil	2023	2020/21	Bioassay	Santo Augusto	RS	1b, 1d, 1k, 2, 3a, 3b, 5, 6, 7	9	27361	MLG 7	Batista et al. 2023
Ps029	Soil	2023	2020/21	Bioassay	Santo Augusto	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	12	27773	MLG 7	This study
Ps030	Soil	2023	2020/21	Bioassay	Guarapuava	PR	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	13	77573	MLG 48	Batista et al. 2023
Ps031	Soil	2023	2020/21	Bioassay	Guarapuava	PR	1a, 1b, 1c, 1d, 1k, 2, 6	7	77040	MLG 69/60	Batista et al. 2023
Ps032	Soil	2023	2020/21	Bioassay	Guarapuava	PR	1a, 1b, 1c, 1d, 1k, 2, 3a, 4, 5, 6, 7	11	77171	MLG 57	Batista et al. 2023

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
Ps033	Soil	2023	2020/21	Bioassay	Senador Salgado Filho	RS	1b, 1d, 2, 3a, 3c, 4, 8	7	25512	MLG 68	Batista et al. 2023
Ps034	Soil	2023	2020/21	Bioassay	Sarandi	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	12	27773	MLG 36/93	This study
Ps035	Soil	2023	2020/21	Bioassay	Senador Salgado Filho	RS	1b, 1d, 3a	3	21100	MLG 66	Batista et al. 2023
Ps036	Soil	2023	2020/21	Bioassay	Caçapava do Sul	RS	1b, 1d, 2, 3a, 3c, 5, 6	7	25560	MLG 29	Batista et al. 2023
Ps037	Soil	2023	2020/21	Bioassay	Caçapava do Sul	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7	12	77571	MLG 51	Batista et al. 2023
Ps038	Soil	2023	2020/21	Bioassay	Caçapava do Sul	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 29	This study
Ps039	Soil	2023	2020/21	Bioassay	Caçapava do Sul	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	13	77573	MLG 46	Batista et al. 2023
Ps040	Soil	2023	2020/21	Bioassay	Caçapava do Sul	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	14	77773	MLG 50	This study
Ps041	Soil	2023	2020/21	Bioassay	Caçapava do Sul	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 28	This study
Ps042	Soil	2023	2020/21	Bioassay	Dom Pedrito	RS	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573	MLG 19	Batista et al. 2023
Ps043	Soil	2023	2020/21	Bioassay	Dom Pedrito	RS	1b, 1d, 2, 3a, 4, 5, 6	7	25170	MLG 6	This study
Ps044	Soil	2023	2020/21	Bioassay	Dom Pedrito	RS	1b, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	26773	MLG 6	This study

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
Ps045	Soil	2023	2020/21	Bioassay	Senador Salgado Filho	RS	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573	MLG 67	Batista et al. 2023
Ps046	Soil	2023	2020/21	Bioassay	Senador Salgado Filho	RS	1b, 1d, 2, 3a, 3c, 5, 6, 7, 8	9	25563	MLG 68	Batista et al. 2023
Ps047	Soil	2023	2020/21	Bioassay	Senador Salgado Filho	RS	1b, 1d, 2, 3a, 3c, 5, 6, 7, 8	9	25563	MLG 68	This study
Ps048	Soil	2023	2020/21	Bioassay	Santa Margarida do Sul	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	12	27773	MLG 18	This study
Ps049	Soil	2023	2020/21	Bioassay	Santa Margarida do Sul	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 20	This study
Ps050	Soil	2023	2020/21	Bioassay	Guarapuava	PR	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	13	77573	MLG 54	This study
Ps051	Soil	2023	2020/21	Bioassay	Guarapuava	PR	1a, 1b, 1c, 1d, 1k, 2, 3a, 3c, 4, 5, 6, 7, 8	13	77573	MLG 53	This study
Ps052	Soil	2023	2020/21	Bioassay	Caçapava do Sul	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 1	This study
Ps053	Soil	2023	2020/21	Bioassay	Alegrete	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 21	This study
Ps054	Soil	2023	2020/21	Bioassay	Dom Pedrito	RS	1c, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	45773	MLG 6	This study
Ps055	Soil	2023	2020/21	Bioassay	Dom Pedrito	RS	1b, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	12	27773	MLG 6	This study

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
Ps056	Soil	2023	2020/21	Bioassay	São Borja	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 6	This study
Ps057	Soil	2023	2020/21	Bioassay	São Borja	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 6	This study
Ps058	Soil	2023	2020/21	Bioassay	São Borja	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 7	This study
Ps059	Soil	2023	2020/21	Bioassay	São Borja	RS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 5	This study
Ps060	Soil	2023	2020/21	Bioassay	Bela Vista	MS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 4	This study
Ps061	Soil	2023	2020/21	Bioassay	Tapes	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	14	77773	MLG 14	This study
Ps062	Soil	2023	2020/21	Bioassay	Tapes	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	14	77773	MLG 13	This study
Ps063	Soil	2023	2020/21	Bioassay	Tapes	RS	1a, 1b, 1c, 1d, 1k, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	14	77773	MLG 23	This study
Ps064	Soil	2023	2020/21	Bioassay	Londrina	PR	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 11	This study
Ps065	Soil	2023	2020/21	Bioassay	Londrina	PR	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 11	This study
Ps066	Soil	2023	2020/21	Leaf-bait	Londrina	PR	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573	MLG 58	This study

Isolate	sample	Study_year	Season	Bait	Municipality	State	Pathotype	Complexities	Octal	MLG	Reference
Ps067	Soil	2023	2020/21	Leaf-bait	Londrina	PR	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573	MLG 58	This study
Ps068	Soil	2023	2020/21	Leaf-bait	Londrina	PR	1b, 1d, 2, 3a, 3c, 4, 5, 6, 7, 8	10	25573	MLG 94	This study
Ps069	Soil	2023	2022/23	Bioassay	Palotina	PR	1d, 2, 3a, 3c, 4, 5, 6, 7, 8	9	05573	NA	This study
Ps070	Soil	2023	2022/23	Bioassay	Palotina	PR	-	-		NA	This study
Ps071	Soil	2023	2022/23	Bioassay	Palotina	PR	1d, 2, 3a, 4, 5, 6, 7, 8	8	05173	NA	This study
Ps072	Soil	2023	2022/23	Bioassay	Palotina	PR	1d, 2, 3a, 3c, 4, 5, 6, 7, 8	9	05573	NA	This study
Ps073	Soil	2023	2022/23	Leaf-bait	Cândido Mota	SP	1d, 3a, 3c, 4, 5, 6, 7	7	01571	MLG 82	This study
Ps074	Soil	2023	2022/23	Leaf-bait	Cândido Mota	SP	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7	10	25771	MLG 26	This study
Ps075	Soil	2023	2022/23	Leaf-bait	Bela Vista	MS	1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7, 8	11	25773	MLG 9	This study

General conclusion

This thesis investigated the interactions between soybean seeds and seedlings with oomycetes, focusing specifically on *Pythium*-like and *Phytophthora sojae*.

Chapter 1 highlighted the underestimated presence of different *Pythium*-like species and *P. sojae*, identifying their significant threat to soybean crops. Five *Pythium* species (*Pythium angustatum*, *P. aphanidermatum*, *P. deliense*, *P. myriotylum* and *P. oopapilum*), four *Globisporangium* species (*Globisporangium acanthophoron*, *G. irregulare*, *G. ultimum*, and *G. ultimum* var. *sporangiiferum*), one *Phytophthora* species (*Phytophthora sojae*), one *Phytopythium* species (*Phytopythium cucurbitacearum*), and one *Aphanomyces* species (*Aphanomyces cladogamus*) were recovered and identified. Pathogenicity tests and fungicide efficacy offered valuable insights for management strategies.

Chapter 2 identified rapid adaptation in the *P. sojae* population. With 28 distinct pathotypes among 40 isolates, the study emphasized increasing complexity and the need for the strategic deployment of the resistance genes.

Exploring genetic evolution over a decade, chapter 3 revealed that the genetic diversity of the *P. sojae* population has remained remarkably consistent. Contrary to expectations of a dominant genotype emerging, the population exhibits a sustained high level of genetic variability. This characteristic of high genetic variability persists, highlighting the need for continued vigilance in the face of the pathogen's evolving dynamics.

The results have significant implications for genetic improvement programs and the development of sustainable strategies to preserve soybean production in Brazil, one of the leading global producers.