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The Biology and Host Range of Phytophthora capsici: An Oomycete Pathogen

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Abstract: -

Phytophthora capsici is a highly adaptable and destructive pathogen affecting a wide range of vegetables. It infects all cucurbits, pepper, tomato, and eggplant, and has recently been reported in snap and lima beans. Over the past few decades, the incidence and severity of the disease have risen significantly. Concurrently, molecular resources for studying this pathogen have expanded, now including a reference genome. The epidemiology of P. capsici varies based on geographical location. In South America, the pathogen primarily reproduces clonally, whereas in the USA and South Africa, it exhibits high genetic diversity due to frequent sexual reproduction. As crop losses caused by *P. capsici* have escalated, there has been a parallel growth in the development of new research tools and resources to better understand and manage the pathogen. Given its broad host range, *P. capsici* serves as an important model for studying oomycete biology, the role of sexual recombination in field populations, and the mechanisms of virulence in *Phytophthora* species.

Introduction

The filamentous oomycete pathogen *Phytophthora capsici* is responsible for root, crown, foliar, and fruit rot in several economically significant vegetable crops. It was first identified in 1922 after being isolated from chili pepper plants at the New Mexico Agricultural Experiment Station in 1918. Initially believed to be host-specific to pepper, it was later found infecting tomato, eggplant, cucurbits (such as cucumber, melon, and pumpkin), and more recently, green and lima beans. The long-distance spread of *P. capsici* remains unclear, but the pathogen has been reported across North and South America, Asia, Africa, and Europe**.** Due to its ability to infect a wide range of plant families,

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researchers have increasingly focused on studying its epidemiology, genetics, and mechanisms of infection and virulence. These studies have revealed that, in many locations, *P. capsici* follows a unique life cycle, frequently utilizing both sexual and asexual reproduction to adapt and spread effectively.

Phytophthora Capsici Taxonomy

Recent genus-wide phylogenetic studies have classified *Phytophthora* species into ten major clades. A closely related sister species with a slightly more basal lineage is *P. tropicalis*. Initially, these two species were considered conspecific due to their morphological similarities and isozyme data. Both species produce deciduous, oblong-shaped sporangia with prominent apical papillae on long pedicels and require outcrossing to form amphigynous oospores. However, isozyme analysis is not sensitive enough to differentiate them clearly, and DNA sequencing has been instrumental in establishing their distinction. Apart from genetic differences, there are biological variations between *P. capsici* and *P. tropicalis*. *P. capsici* is primarily recovered from vegetables and does not produce abundant chlamydospores in culture, whereas *P. tropicalis* is predominantly found in woody nursery and perennial crops such as black pepper and cacao. While most hybridization attempts between the two species have failed, one successful in vitro cross resulted in oospore progeny carrying alleles from both parents. In Tennessee, *P. tropicalis* is frequently isolated from nursery-grown ornamentals but has never been recovered from nearby vegetable fields. Conversely, *P. capsici* has not been found in nurseries. Despite their distinct characteristics, misidentification persists due to morphological similarities and the presence of mislabelled internal transcribed spacer (ITS) sequences in GenBank.

Epidemiology and population biology of *P. Capsici*

The epidemiology and population structure of *Phytophthora capsici* have been examined in various regions worldwide, providing valuable insights into its population biology. In South Africa and the United States, both mating types are present, and populations exhibit high levels of genotypic and genetic diversity, likely due to frequent sexual reproduction. In contrast, studies of over 200 field isolates from pepper-growing regions along the Peruvian coast identified only a single mating type (A2) and a limited number of genotypes, indicative of clonal lineages. One particular clonal lineage was predominant, spanning a vast geographical range and persisting across multiple growing seasons, suggesting an absence of sexual reproduction. A similar pattern has been

observed in Argentina, where all *P. capsici* isolates belong to the A1 mating type, and a single clonal lineage dominates across large areas and multiple years. These findings from Peru and Argentina sharply contrast with those from the USA and South Africa, where both mating types are prevalent and populations show significant genetic diversity. While the reason for these fundamental epidemiological differences remains uncertain, it is hypothesized that continuous crop production throughout the year may negate the necessity for sexual reproduction and oospore formation, which typically help the pathogen survive between growing seasons. The fact that these clonal isolates remain fertile under laboratory conditions further supports this hypothesis.

Resistance to *P. Capsici* and management

So far, resistance to *Phytophthora capsici* has been identified in only a few specific crops. In pepper, genetic studies have led to the discovery of several major quantitative trait loci (QTLs) associated with resistance. However, the only known source of broad resistance against all tested *P. capsici* isolates is found in the pepper landrace *Criollo de Morelos-CM334*, which is not commercially available. The development of resistant commercial cultivars remains a challenge due to the genetic diversity of *P. capsici* isolates, making it unlikely that resistant pepper varieties will be introduced in the near future. In tomatoes, an extensive screening process using four different *P. capsici* isolates identified one resistant accession, *Solanum habrochaites* (LA407), which demonstrated resistance to all four strains tested. However, further studies will be necessary to determine whether the genetic diversity of *P. capsici* populations will eventually overcome the resistance observed in both pepper and tomato. Understanding the molecular mechanisms behind this resistance will be crucial for growers, plant breeders, and researchers. Once *P. capsici* is established in a location, controlling it becomes extremely difficult. Most management strategies focus on minimizing losses by reducing excess water. This includes practices such as selecting well-drained planting sites, using raised beds, implementing controlled irrigation methods (e.g., trickle irrigation), and, where feasible, growing crops on trellises. Unfortunately, no chemical or cultural method currently exists to effectively control the disease under warm and wet conditions.

***Phytophthora Capsici* reference genome sequence**

Given the biological complexity and high level of polymorphism within *Phytophthora capsici* field populations, a genome sequencing project for *P. capsici* was initiated in 2005. The objective was to create a draft reference genome sequence and establish

a single nucleotide polymorphism (SNP) database. To address the difficulties of assembling a highly polymorphic genome, researchers conducted a series of inbreeding crosses, ultimately selecting a moderately inbred isolate for sequencing. The initial cross involved two field isolates: an A2 mating type from Tennessee (LT263), recovered from pumpkin in 2004, and an A1 mating type from Michigan (LT51), recovered from cucumber in 1997. Both parental isolates exhibited high fecundity, formed dense oospore mats upon mating, and produced a high percentage of viable, recombinant oospores. After the initial cross, two successive backcrosses were performed using LT263 as the recurrent parent. From the resulting progeny, isolate LT1534 (A2 mating type) was selected based on key characteristics, including heavy and spontaneous sporulation on V8 juice agar, robust oospore production when crossed with A1 *P. capsici* isolates, and abundant zoospore production from sporangial preparations. Subsequent sequencing of LT1534 using traditional Sanger sequencing along with next-generation sequencing (NGS) technologies (454 + Illumina) resulted in a draft genome covering 64 MB of the estimated 65-MB *P. capsici* genome (98.4% coverage). The reference genome (*Phyca11*) is publicly available at JGI. This high-quality genome has significantly advanced research on *P. capsici* biology, shedding light on infection mechanisms, genetic diversity, and effector evolution.

Phytophthora Capsici SNP Data Resources

Analyses using various anonymous molecular markers-including amplified fragment length polymorphisms (AFLPs), random amplification of polymorphic DNA (RAPD), simple sequence repeats (SSRs), and isozymes-have revealed that many *Phytophthora capsici* populations exhibit significant genotypic diversity. Unsurprisingly, genome sequencing projects are uncovering even higher levels of genetic diversity. Initial estimates, based on the re-sequencing of 20 single-copy nuclear genes (approximately 11 kb in total) from four *P. capsici* isolates originating from Peru, Michigan, Tennessee, and New York, indicated a SNP substitution rate of one polymorphic site per 40 bases (K. Lamour, unpublished data). Ongoing research aims to identify and validate a comprehensive panel of SNP markers for *P. capsici* using progeny from an in vitro cross. This process utilizes a targeted re-sequencing technique known as restriction site-associated DNA (RAD) sequencing, which reduces costs by: (i) restricting sequencing to regions adjacent to a restriction enzyme cut site, and (ii) incorporating molecular tags that allow pooled DNA samples to be sequenced on a next-generation platform. For *P. capsici*, genomic

DNA was extracted from LT263 and LT51 (the parental strains of the sequenced isolate LT1534), along with 60 progeny derived from these parents. The DNA samples were digested with the restriction enzyme *PstI*, tagged, pooled, and sequenced using a GAII analyzer. Given that the *P. capsici* genome is predicted to contain approximately 30,000 *PstI* restriction sites, the RAD sequencing data provided 10× coverage across 5 MB of reference sequence between the parents, offering a broad perspective on SNP distribution (K. Lamour, unpublished results). Preliminary findings indicate a high level of heterozygosity in the parental isolates, with a segregating SNP occurring every 200 base pairs. The validation and mapping of these markers will create a valuable resource for investigating key questions, such as identifying genes involved in pathogenicity, virulence, and reproduction, as well as pinpointing regions of the genome under positive selection.

Insights into *P. capsici* effector biology

The availability of a draft *Phytophthora capsici* genome has significantly accelerated research on the molecular mechanisms driving infection. Basic bioinformatic tools now allow for the rapid identification of proteins that have been extensively studied in other *Phytophthora* species, enabling their use as marker genes in comparative analyses. Through this approach, the initial biotrophic phase, the subsequent transition to necrotrophy, and the onset of sporulation have been confirmed across various host plants (E. Huitema, unpublished results). Further investigations into *P. capsici*–host interactions are ongoing.

Plant pathogens, including *Phytophthora* species, secrete a diverse array of effector proteins that facilitate infection and reproduction. In the early stages of infection, affected plant cells appear healthy, reflecting a biotrophic phase. During this phase, a class of effectors known as RXLRs is secreted and delivered across the haustorial interface into host cells, where they are believed to promote infection. These effectors are characterized by a signal peptide followed by a conserved RXLR motif, which enables their rapid identification from oomycete genome sequences. A genome-wide search of the *P. capsici* draft genome identified over 400 putative RXLR genes, highlighting their likely role in *P. capsici* pathogenicity (E. Huitema, unpublished results). Gene expression analysis of a subset of RXLR candidates confirmed their association with the biotrophic phase, supporting their role in virulence. Future research aims to further elucidate the precise functions of RXLR effectors in *P. capsici*–host interactions. Beyond RXLRs, another class of cytoplasmic effectors, known as "crinklers"

(CRNs), has recently been characterized in *Phytophthora*. The Crn1 and Crn2 proteins were initially identified in a high-throughput screen of secreted *P. infestans* proteins, named after their ability to induce "crinkling and necrosis" (CRN) in plants. CRN proteins share a conserved N-terminal region with a signal peptide and a highly conserved LQLFLAK motif essential for translocation. Their C-terminal effector domains are highly diverse and are thought to contribute to virulence. Computational analysis of the *P. capsici* genome identified approximately 80 full-length CRN genes and more than 200 pseudogenes, suggesting rapid evolution. Further studies on a subset of CRN effectors indicate that, like their *P. infestans* counterparts, *P. capsici* CRN effectors target the host nucleus, potentially reprogramming host cells to facilitate infection (E. Huitema, unpublished results). Ongoing research seeks to uncover the host processes and targets affected by these effectors. In addition to intracellular effectors, recent studies have identified other secreted proteins as potential virulence factors. In *P. capsici* strain SD33, 18 PcNpp (necrosis-inducing *Phytophthora* protein) and nine pectin methylesterase (Pme) genes were discovered. NPP genes in other oomycetes have been linked to host cell death, suggesting a role in disrupting host cells during infection. Structural studies on *Pythium aphanidermatum* NPP-like proteins revealed similarities to cytolytic actinoporins, indicating possible pore-forming activity during infection. Given their high expression levels and ability to induce cell death, NPP proteins are thought to contribute to the transition from biotrophy to necrotrophy. Although 12 PcNpp genes were found to be expressed during infection, their precise roles in *P. capsici* virulence remain unclear. Similarly, PcPme genes were also found to be expressed during infection, and exposure of plant tissue to PcPME resulted in tissue collapse and cell death. This suggests that their mechanism of action and role in virulence may differ from that of NPP proteins. Further functional studies are necessary to clarify the contributions of these proteins to *P. capsici* pathogenicity.

CONCLUSIONS

As crop losses caused by *P. capsici* have risen in recent decades, there has been a corresponding expansion in tools and resources available to study this destructive pathogen. The ease of working with *P. capsici*, along with its reference genome and extensive molecular diversity, offers a valuable framework for investigating some of the most complex aspects of *Phytophthora* parasitism and evolution. Specifically, *P. capsici* serves as a useful model for examining broad-host-range oomycete pathogens, the influence of sexual recombination in field populations, the

development of clonal lineages, and the fundamental mechanisms of *Phytophthora* pathogenicity.

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