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Phytophthora capsici: Diversidade e resistência em *Solanum* (*Lycopersicon*)

DÉBORA GONÇALVES PEREIRA

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Phytophthora capsici: Diversidade e resistência em *Solanum* (*Lycopersicon*)

Tese apresentada à Universidade de Brasília como requisito parcial para a obtenção do título de Doutor em Fitopatologia pelo Programa de Pós-Graduação em Fitopatologia

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PHYTOPHTHORA CAPSICI: DIVERSIDADE E RESISTÊNCIA EM _SOLANUM_ (_LYCOPERSICON_).

DÉBORA GONÇALVES PEREIRA

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O oomiceto *Phytophthora capsici* Leonian (Peronosporales, Pythiaceae) pode induzir perdas severas em várias culturas, incluindo o tomateiro (*Solanum lycopersicum* L.). O **capítulo I** apresenta uma revisão sobre *P. capsici* e a dificuldade de manejo desse patógeno devido à ausência de cultivares resistentes e pela grande variação no perfil de virulência dos isolados. Apesar da importância econômica de *P. capsici* no tomateiro, poucos trabalhos têm estudado fontes de resistência bem como os padrões de interação entre acessos de *Solanum* (*Lycopersicon*) e isolados neotropicais deste patógeno. No **capítulo II** são apresentados resultados sobre a estrutura genética de 45 isolados classificados como *P. capsici*. Posteriormente, dez isolados de diferentes hospedeiras foram avaliados quanto a sua capacidade de causar doença em frutos de pimentão e plântulas de tomateiro cultivar 'Santa Clara' e pimentão cultivar 'Tico'. Isolados de diferentes estados do Brasil, e diferentes hospedeiras foram genotipados para o gene *cox*2. Todos os 45 isolados avaliados com *primer* específico produziram amplicons de tamanho esperado. Os dois grupos de compatibilidade (A1 e A2) foram observados entre os isolados, mesmo entre coletas do mesmo local. Os isolados 'PCa-31', 'PCp-183', 'PCp-167', 'PCa-29', 'PCa-31', 'PCp-183', 'PCp-167' e 'PCa-29' causaram doenças nos frutos de pimentão, sendo possível visualizar micélio sobre o tecido infectado, quatro dias após a inoculação (DAI). Alguns isolados que causaram doença em frutos de pimentão, não causaram doença em plântulas de 'Tico' e 'Santa Clara', sugerindo que existe interação do tipo tecido-específica para a indução de sintomas. A análise das sequências do *cox*2 obtidas dessa coleção de isolados, indicaram que esse locus é eficiente como "barcoding" para identificação de isolados de *P. capsici*. As relações filogenéticas entre isolados para o gene *cox*2 não exibiram agrupamentos relacionados com os grupos de compatibilidades, local de coleta ou planta hospedeira original. No **capítulo III** foram apresentados resultados de três bioensaios controlados conduzidos com o objetivo de avaliar a reação de 28 acessos de *Solanum* (*Lycopersicon*) contra uma coleção de sete isolados de *P. capsici*. *Capsicum annuum* 'Tico' foi usado como controle suscetível. As inoculações foram realizadas via deposição de uma suspensão (2×10^4 zoósporos por mL) ao redor do colo das mudas. A taxa de mortalidade foi avaliada aos 14 DAI. Todos os isolados (em todos os bioensaios) induziram sintomas severos em 'Tico' (100% de mortalidade). A linhagem *S.*

lycopersicum ‘Hawaii 7996’ apresentou níveis superiores de resistência do tipo isolado-específica para quatro de seis isolados, enquanto *S. habrochaites* ‘WIR 7924’ exibiu resistência a cinco de sete isolados. Dois dos 18 acessos de *S. habrochaites* (‘PI 127826’ e ‘PI 127827’) apresentaram resistência do tipo imunidade contra dois isolados de *P. capsici*. As respostas de resistência desses acessos não foram coincidentes, indicando a presença potencial de patótipos. Respostas instáveis de alguns acessos foram observadas nos ensaios, indicando herança complexa ou penetrância incompleta da resistência. O desenvolvimento de cultivares com amplo espectro de resistência a múltiplos isolados de *P. capsici* é essencial para o manejo sustentável desse patógeno oomiceto altamente variável. Portanto, piramidar fatores de resistência de ‘Hawaii 7996’ e de acessos de *S. habrochaites* em único genoma seria uma estratégia de melhoramento promissora visando desenvolver cultivares de tomate com resistência estável contra uma ampla gama de isolados de *P. capsici.* Extensa variação no perfil de virulência tem sido observada para muitos isolados do patógeno, induzindo reações contrastantes entre acessos de plantas hospedeiras. No entanto, nenhum trabalho mais extenso investigou os padrões de interação entre *Solanum* (*Lycopersicon*) e isolados do patógeno. No **capítulo IV** foram conduzidos estudos visando identificar a presença de potenciais patótipos bem como a definição de um painel adequado de acessos de hospedeiros diferenciais nesse patossistema. Dezessete isolados virulentos foram utilizados para avaliar acessos de *Solanum* (*Lycopersicon*) que apresentaram níveis superiores de resistência a um ou mais isolados em bioensaios anteriores. Oito potenciais patótipos foram identificados de acordo com seus padrões de interação com nove acessos de *Solanum* (*Lycopersicon*). A cultivar *S. lycopersicum* ‘Santa Clara’ apresentou uma reação suscetível “universal” (100% de mortalidade para todos os isolados), enquanto ‘Hawaii 7996’ seguida por *S. habrochaites* ‘PI 127826’ e ‘PI 127827’ foram as principais fontes de amplo espectro de resistência, exibindo desempenho superior contra ampla gama de isolados. Estudos de herança e mapeamento desses fatores de resistência do tipo patótipo-específica de cada acesso facilitarão sua incorporação em cultivares comerciais. Esses oito acessos informativos de *Solanum* (*Lycopersicon*) são sugeridos como acessos de hospedeiros diferenciais para esse patossistema e podem fornecer um panorama mais preciso dos patótipos que ocorrem em campo por meio de bioensaios simples baseados na capacidade dos isolados de “quebrar” esse conjunto único de genes específicos deste germoplasma. No **capítulo V** foram conduzidos estudos de herança para determinar a base genética da resistência a isolados de *P. capsici* detectada no acesso *S. habrochaites* ‘PI 127827’. Cruzamentos foram efetuados com a acesso

suscetível ‘Ponderosa’. Populações F_1 e F_2 foram obtidas e inoculadas com suspensão de zoósporos do isolado ‘PCp-182’. A distribuição de frequência de indivíduos resistentes e suscetíveis (avaliada pelo teste qui-quadrado) indicou bom ajuste para modelo epistático (15:1) envolvendo dois fatores genéticos dominantes em duplicata. Desta forma, as informações geradas no presente trabalho fornecem elementos cruciais para o estabelecimento de uma base cientifica e tecnológica para o desenvolvimento de cultivares de tomateiro com resistência estável e durável contra um patógeno com amplo círculo de plantas hospedeiras.

Palavras-chave: raças, resistência, melhoramento genético, oomiceto, virulência, zoósporos.

Comitê de orientação: Leonardo Silva Boiteux – Embrapa Hortaliças, Ailton Reis – Embrapa Hortaliças, Maria Esther de Noronha Fonseca Boiteux – Embrapa Hortaliças.

GENERAL ABSTRACT

Gonçalves-Pereira, Débora. ***Phytophthora capsici*: Pathogen diversity and resistance in *Solanum (Lycopersycon)*.** 2023. (192p). Thesis (Doctorate in Plant Pathology) – Universidade de Brasilia, Brasilia, DF, Brazil.

The oomycete *Phytophthora capsici* Leonian (Peronosporales, Pythiaceae) can induce severe losses in several crops, including tomato (*Solanum lycopersicum*). **Chapter I:** Provides a review of *P. capsici* and how difficult is the management of this oomycete, mainly due to the lack of resistant cultivars and the wide variation in the virulence profile of the isolates of the pathogen. Although the economic importance of *P. capsici* for the tomato crop, few studies have studied sources of resistance as well as interaction patterns of *Solanum* (*Lycopersicon*) accessions and neotropical isolates of this pathogen. In **Chapter II**, the genetic structure of 45 isolates was investigated in isolates previously classified as *P. capsici*. Subsequently, ten isolates from different hosts were evaluated for their ability to cause disease in sweet pepper fruits and seedlings of the tomato cultivar 'Santa Clara' and of the bell-pepper cultivar 'Tico'. Isolates from different Brazilian states and hosts were genotyped for *cox*2 gene. All 45 isolates evaluated with primer-specific produced amplicons of the expected size. The two compatibility groups (A1 and A2) were observed among the isolates, even among collections from the same location. The isolates 'PCa-31', 'PCp-183', 'PCp-167', 'PCa-29', 'PCa-31', 'PCp-183', 'PCp-167', and 'PCa-29' were able to induce symptoms in bell-pepper fruits, being possible to visualize mycelium on the infected tissue, four days after inoculation. Some isolates that caused symptoms in bell-pepper fruits did not cause symptoms in seedlings of 'Tico' and 'Santa Clara', suggesting a tissue-specific interaction for induction of symptoms. The analysis of the *cox*2 sequences obtained from this collection of isolates indicated that this locus is efficient as "barcoding" for identification of *P. capsici* isolates. The phylogenetic relationships observed among isolates for the *cox*2 gene did not show clusters according to either the compatibility groups, collection site or original host plant. **In Chapter III:** three controlled bioassays were conducted to evaluate the reaction of 28 accessions of *Solanum* (*Lycopersicon*) against a collection of seven *P. capsici* isolates. *Capsicum annuum* 'Tico' was used as a susceptible control. Inoculations were performed by depositing a suspension (2 x 10^4 zoospores per mL) around the crown area of the seedlings. The mortality rate was evaluated 14 days after inoculation. All isolates (in all bioassays) induced severe symptoms in 'Tico' (100% mortality). The accession *S. lycopersicum* 'Hawaii 7996' displayed superior

levels of isolate-specific resistance to four out of six isolates, while *S. habrochaites* 'WIR 7924' exhibited resistance to five out of seven isolates. Two of the 18 accessions of *S. habrochaites* ('PI 127826' and 'PI 127827) displayed immunity-like resistance against two *P. capsici* isolates. The resistance responses of these accessions were not coincident, indicating the potential presence of pathotypes. Unstable responses of some accessions were observed in the trials, indicating complex inheritance or incomplete penetrance of the resistance. The development of cultivars with a broad-spectrum of resistance to multiple *P. capsici* isolates is essential for the sustainable management of this highly variable pathogenic oomycete. Therefore, pyramiding resistance factors from 'Hawaii 7996' and *S. habrochaites* accessions into a single genome would be a promising breeding strategy aimed at developing tomato cultivars with stable, broad-spectrum resistance to a wide range of *P. capsici* isolates. Extensive variation in the virulence profile has been observed for many pathogen isolates, inducing sharp contrasting reactions among host accessions. However, no extensive work has investigated the interaction patterns between *Solanum* (*Lycopersicon*) and pathogen isolates. In **Chapter IV**, studies were conducted to identify the presence of potential pathotypes as well as the definition of an adequate panel of accessions of differential hosts in this pathosystem. Seventeen virulent isolates (from different host plants and geographic regions) were used to evaluate accessions of *Solanum* (*Lycopersicon*) that showed superior levels of resistance to one or more isolates in previous bioassays. Eight potential pathotypes were identified according to their interaction patterns with nine accessions of *Solanum* (*Lycopersicon*). The cultivar *S. lycopersicum* 'Santa Clara' exhibited a "universal" susceptible reaction (100% mortality for all isolates), while 'Hawaii 7996' followed by *S. habrochaites* 'PI 127826' and 'PI 127827' were the main sources broad-spectrum resistance, exhibiting superior performance against a wide range of isolates. Inheritance studies and mapping of these pathotype-specific resistance factors for each accession will facilitate their incorporation into commercial cultivars. These eight informative accessions of *Solanum* (*Lycopersicon*) are suggested as differential host accessions for this pathosystem and may provide a more accurate picture of the pathotypes that occur in the field through simple bioassays based on the capacity of the isolates to "breakdown" this unique set of specific genes of this germplasm. In **Chapter V**, inheritance studies were carried out to determine the genetic basis of resistance to *P. capsici* identified in the accession *S. habrochaites* 'PI 127827' resistance. Crosses were made with the susceptible accession 'Ponderosa'. The F1 and F2 populations were obtained and inoculated with a zoospore suspension of isolate 'PCp-182'. The frequency

distribution of resistant and susceptible plants in the F_2 generation (evaluated by the Chi-square test) indicated a good fit for an epistatic model (15:1) involving two dominant genetic factors in duplicate. In summary, the information generated in the present work provides crucial elements for the establishment of a scientific and technological base for the development of tomato cultivars with stable and durable resistance against a pathogen with a wide range of host plants.

Keywords: races, resistance, genetic improvement, oomycete, virulence, zoospores.

Guidance Committee: Leonardo Silva Boiteux – Embrapa Hortaliças, Ailton Reis – Embrapa Hortaliças, Maria Esther de Noronha Fonseca Boiteux – Embrapa Hortaliças.

INTRODUÇÃO GERAL

A podridão de raiz, colo e o tombamento de mudas, causados pelo oomiceto *Phytophthora capsici*, é um complexo de doenças de maior importância no cultivo do tomateiro em diversas regiões geográficas. A rotação com não hospedeiras, controle químico, biológico, cultural e/ou físico são abordagens amplamente aplicadas em áreas infestadas por *P. capsici*, no entanto, algumas práticas têm sido ineficientes frente à diversidade de mecanismos evolutivos gerados pela recombinação genética, migração, seleção e existência de estruturas de resistência que podem sobreviver na ausência de hospedeiras por longos anos. Além disso, é válido ressaltar que não existem fontes de resistência em variedades comerciais o que demonstra a necessidade de compreensão do patossistema *P. capsici-Solanum lycopersicum* a fim de desenvolver variedades agronomicamente produtivas e resistentes.

A correta identificação de *P. capsici* é crucial para o manejo eficiente das doenças em diferentes culturas. A caracterização morfológica considera aspectos como comprimento de pedicelos, esporângios, caducidade, inserção do anterídeo, sistema reprodutivo, ausência ou presença de papila no esporângio, entretanto, leva a classificações precipitadas, uma vez que, existem homoplasias interespecíficas, expressando a necessidade de adoção de técnicas mais robustas e eficientes no nível de espécie. O sequenciamento de regiões genômicas é eficaz quanto à caracterização molecular dessa espécie, uma vez que consegue separar espécies evolutivamente próximas como *P. tropicallis*, e agrupar espécies morfologicamente diferentes como *P. mexicana.* O uso de tecnologias moleculares possibilita o aperfeiçoamento da interpretação de resultados obtidos ao longo de anos e permite a formulação de hipóteses importantes no desenvolvimento de pacotes de manejo desse complexo de doenças. Nesse sentido, a escolha de genes para identificação de *P. capsici* é um desafio frente à quantidade de opções disponíveis nos bancos de dados, afirmando a existência de interesse da

comunidade científica quanto à compreensão das forças evolutivas desse fitopatógeno que está inserido em um dos principais gêneros causadores de perdas econômicas na agricultura.

A existência de diferentes grupos de compatibilidade gera estruturas de resistência, na forma de oósporos com paredes espessas que sobrevivem no solo por longos anos. Outrossim, a possibilidade de reprodução sexual leva ao aumento da variabilidade genética que pode levar a insensibilidade à oomeceticidas. Também existem fontes de variação, nas populações clonais por meio de mutações, recombinação mitótica, recombinação parassexual e hibridação interespecífica. Nesse viés, já foi relatada a hibridação interespecífica entre *P. capsici* e *P. tropicalis*. Em suma, conhecer a estrutura populacional de *P.capsici* é fundamental para o adequado manejo dessa doença.

Existe grande variação no perfil de virulência dos isolados de *P. capsici*, ao considerar-se a interação acesso-isolado. Os perfis de virulência em *P. capsici* são definidos de acordo com a reposta das plantas inoculadas, a origem do isolado e características da estrutura populacional do patógeno. Devido ao alto nível de diversidade encontrado em *P. capsici,* é valiosa a identificação de possíveis patótipos para a adequada seleção de acessos com amplo espectro de resistência. Em *Capsicum* são relatadas diferentes raças, descritas pelos diferentes níveis de virulência em um grupo de plantas diferenciadoras. Em tomateiro ainda não é difundido um conjunto de acessos que permitem a análise de perfis de virulência. Possivelmente, essa variação quanto aos níveis de virulência deve-se a localização dos genes, envolvidos na patogenicidade, estarem em regiões do genoma menos conservadas sob influência de diferentes mecanismos de variação e seleção que levam a rápida adaptação e co-evolução com as hospedeiras.

O tomateiro (*Solanum lycopersicum* L.) é suscetível a muitas doenças dificultando os programas de melhoramento quanto ao desenvolvimento de variedades resistentes. Sabe-se muito pouco sobre a base genética da resistência à *P. capsici* em tomateiro. A resistência em

Capsicum annumm é complexa, podendo ser controlada por poucos ou múltiplos genes dominantes e/ou recessivos, sendo muitas vezes, fortemente influenciada por condições ambientais. Em *Solanum lycopersicum* L. algumas fontes de resistência têm sido descritas em acessos selvagens, entretanto sem a identificação do(s) gene(s) responsável pela resposta de resistência. Nesse sentido, é necessário determinar o controle genético da resistência, para se estabelecer a estratégia mais eficiente para os programas de melhoramento.

Diante desse contexto, os objetivos do presente trabalho foram estudar a base genética e a amplitude da resistência contra isolados de *P. capsici* detectada em acessos do gênero *Solanum* (*Lycopersicon*) previamente identificados como altamente resistentes e compreender a diversidade de isolados de *P. capsici*. Por meio da identificação de novas fontes de resistência de amplo espectro contra isolados de *P. capsici* em acessos de *Solanum* (seção *Lycopersicon)*; Identificar possíveis patótipos em um conjunto de acessos contra isolados da coleção de fungos e oomicetos da Embrapa Hortaliças; Confirmar a identidade e caracterizar isolados de *P. capsici* pertencentes à coleção de fungos e oomicetos da Embrapa Hortaliças; Identificar os grupos de compatibilidade dos isolados; Testar a capacidade de causar doença em frutos de pimentão e plântulas de pimentão Tico. Estudar a herança da resistência número de genes envolvidos na resistência e o tipo de controle (dominância vs. recessividade).

CAPÍTULO I

Revisão de Literatura

1.1. Plantas hospedeiras de *Phytophthora capsici*: Tomateiro e outras espécies de Solanaceae

O tomateiro (*Solanum lycopersicum* L.) é uma das hortaliças mais importantes no mundo, sendo os seus frutos consumidos de diversas formas em diferentes regiões (Foolad, 2007; Campos *et al.,* 2021). Estima-se que 4,6 milhões de hectares são cultivados anualmente com tomateiros para consumo *in natura* e indústria em todo o mundo, produzindo mais de 126 milhões de toneladas (fonte: FAO Statistics; http://faostat3.fao.org/home/index.html). No Brasil, o tomateiro é cultivado em diferentes regiões com produção de 3.614.934 toneladas no ano de 2021 ocupando a 9ª posição na lista de maiores produtores de tomate, sendo a China a primeira colocada (fonte: FAO Statistics; http://faostat3.fao.org/home/index.html).

O centro de origem do tomateiro é o Novo Mundo, uma vez que todas as espécies silvestres são nativas da região andina, composta atualmente por partes do Peru, Chile, Equador e Bolívia. Ademais, algumas espécies (por exemplo, *S. galapagense*) são encontradas nas Ilhas Galápagos (Jenkins, 1948; Rick, 1976; 1978). Apesar da ampla distribuição do gênero *Solanum* na região andina e embora o Peru tenha sido amplamente aceito como centro original do tomateiro, a maior parte das evidências históricas, linguísticas, arqueológicas e etno-botânicas indicam o México como o local inicial de domesticação dessa espécie vegetal (Rick, 1976). Ademais o gênero *Solanum* (seção *Lycopersicon*) é composto pela espécie cultivada *S. lycopersicum*, 12 espécies silvestres (Peralta *et al*., 2005; Peralta *et al*., 2008) e quatro espécies selvagens relacionadas (**Figura 1**). Outros membros da família Solanaceae são a berinjela, pimenta, pimentão e jiló, todas hortaliças de importância econômica e cultural que podem ter sua produção afetada pelas doenças causadas por *P. capsici* (Ichihashi & Sinha, 2014; Saltos *et al*., 2022).

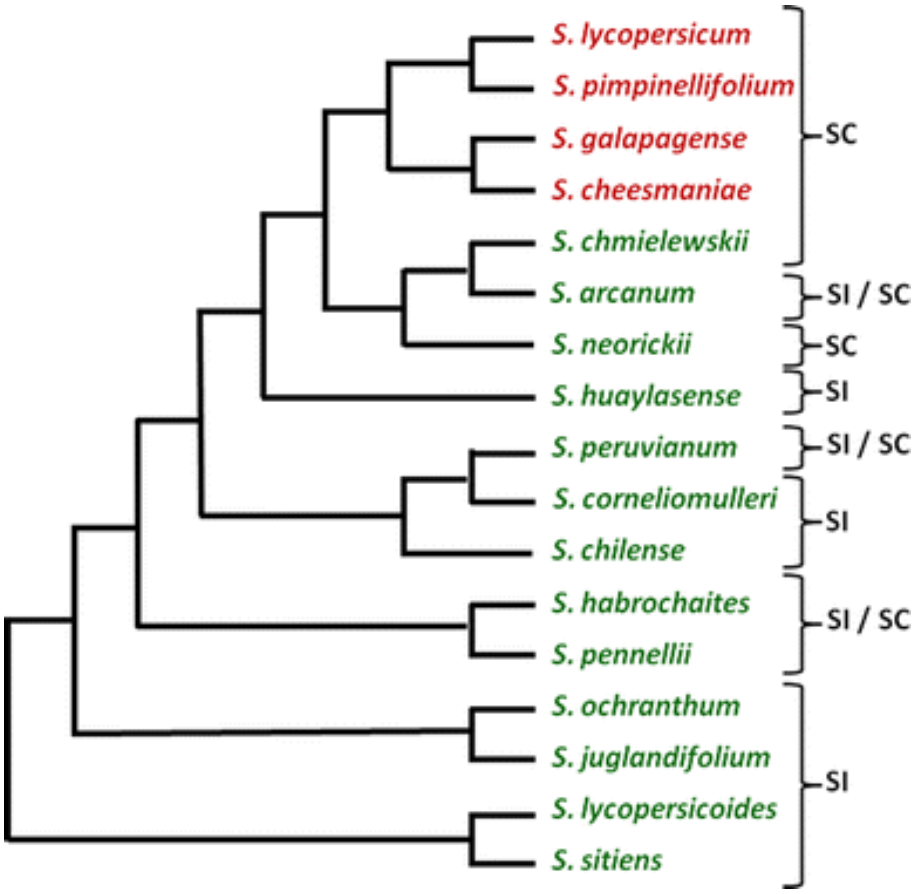

Figura 1. Agrupamentos filogenéticos de espécies de *Solanum* (*Lycopersicon*) de acordo com Rodriguez *et al.* (2009) e Bedinger *et al.* (2011). As quatro espécies em fonte vermelha (*S. lycopersicum*, *S. pimpinellifolium*, *S. galapagense* e *S. cheesmaniae*) produzem frutos vermelhos ou alaranjados quando maduros. As demais espécies (em fonte de cor verde) produzem frutos maduros verdes ou roxos. SC = espécie auto-compatível, SI = auto-incompatível.

1.2 Plantas hospedeiras de *Phytophthora capsici*: Cucurbitáceas

A Cucurbitaceae é a segunda maior família botânica, composta por 115 gêneros e 960 espécies monóicas ou dióicas, distribuídas nas zonas tropicais e subtropicais, raramente encontradas nas zonas temperadas (Ma *et al.*, 2022). As espécies, dessa família botânica, mais conhecidas no Brasil são: melão (*Cucumis melo*), melancia (*Citrullus lanatus*), abobrinha (*Cucurbita pepo*), abóboras (*Cucurbita maxima*, *C. moschata e C. argyrosperma*), pepino

(*Cucumis sativus*), chuchu (*Sechium edule*) e maxixe (*Cucumis anguria*). O centro de domesticação da maioria das espécies de cucurbitáceas foi as Américas, África e Ásia. Nesse sentido, a domesticação de espécies de cucurbitáceas foi provavelmente conduzida por pequenos agricultores que foram selecionando frutos menos amargos e com caracteres morfológicos mais desejáveis (Ma *et al.*, 2022). Por serem frutos consumidos em menor escala que o arroz, trigo, batata e outros alimentos cultivados em grandes áreas, as cucurbitáceas passaram por diversos gargalos durante a domesticação. Além disso, as cucurbitáceas são cultivadas como anuais, entretanto, esse desenvolvimento não é resultado de mudança genética, mas de práticas de manejo para produção de hortaliças, de acordo a demanda do mercado (Chomicki, Schaefer, & Renner, 2020). A China é reponsável por um terço da produção mundial das principais cucurbitáceas (http://www.fao.org/faostat/en/#data/QC) **(Figura 2)**.

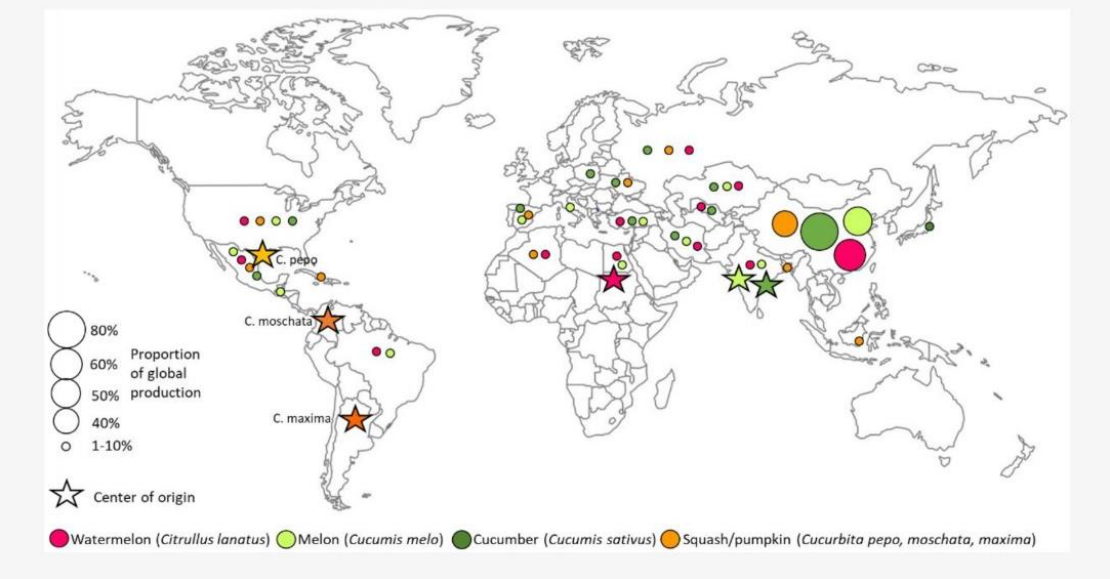

Figura 2. Centro de domesticação de espécies de cucurbutáceas comercializadas em larga escala no mundo, representados por estrela laranja (*Cucurbita pepo, C. moschata e C. máxima*), rosa (*Citrullus lanatus*), verde escuro (*Cucumis sativus*), verde claro (*Cucumis melo*) e níveis de produção dessas espécies representada por círculos (o tamanho de cada círculo é proporcional à produção). (Fonte: Grumet *et al.*, 2021).

Espécies de cucurbitáceas que compõem a dieta humana são nutritivas, saborosas e coloridas. Uma significativa gama de patógenos induz danos consideráveis na produção e na qualidade em diferentes espécies de cucurbitáceas. Nesse viés, *P. capsici* causa podridão dos frutos em melão, pepino, melancia, abobrinha italiana e, principalmente, abóboras (*Cucurbita* spp.). Apesar das cucurbitáceas possuírem grande diversidade genética, apenas um reduzido número de cultivares comerciais apresenta resitência a *P. capsici* (Grumet *et al.,* 2021).

1.3. O patógeno: *Phytophthora capsici*

1.3. 1. Sintomatologia

O oomiceto *Phytopthora capsici* Leonian, pode causar danos durante todo o período de crescimento de espécies de solanáceas, tais como o tomateiro. Esse patógeno causa diversas doenças entre as quais pode-se citar: tombamento de mudas, podridão das raízes, podridão do colo, queima das folhas, amarelecimento, podridão dos frutos e morte da planta (Lamour *et al.*, 2012; Saltos *et al.*, 2022). Os sintomas podem variar consideravelmente de acordo com a hospedeira, órgão da planta infectada e condições ambientais (Granke *et al*., 2012; Barchenger *et al.*, 2018). A infecção no tomateiro e em espécies de *Capsicum* é geralmente observada na parte aérea próxima do colo da planta. Além disso, plantas infectadas têm uma lesão distinta, preta/marrom visível no colo da planta (**Figura 3**). Em áreas em que as chuvas são mais frequentes, todas as partes da planta podem apresentar sintomas, incluindo as raízes, parte aérea, folhas e frutos. Infecções radiculares causam tombamento das plântulas, enquanto em plantas mais velhas, é comum observar podridão do colo, desenvolvimento atrofiado, murcha e, eventualmente, morte. No tomateiro, é comum perceber um crescimento significativo de raízes adventícias. Sendo assim, plantas severamente comprometidas, podem não morrer, no entanto tem sua produtividade reduzida. Em cucurbitáceas, lesões, de grande dimensão são observadas nos frutos. Nessas lesões ocorre profusa produção de esporângios ao longo de dias (ou mesmo semanas, dependendo do tamanho do fruto) e podem ser facilmente

dispersos pela água da irrigação iniciando o processo de infecção em outras plantas. Geralmente, as hifas não emergem de plantas ou frutos infectados (comum nas infecções por *Pythium*) e tudo o que é visível na superfície de uma planta infectada são os esporângios (Hausbeck & Lamour, 2004; Lamour *et al*., 2012; Saltos *et al*., 2022).

Figura 3. Tomateiro apresentando sintoma de tombamento de muda e podridão do colo causado por *P. capsici*. Fonte: Reis, A.(2006).

1.3.2. Epidemiologia

Esse patógeno já foi relatado infectando diversas hospedeiras e apresenta distribuição cosmopolita com relatados por diversos países. Essa ampla distribuição se torna um motivo adicional de preocupação, uma vez que o manejo se torna difícil após o seu ingresso em uma determinada área (Pontes *et al.*, 2014; Barboza *et al*., 2017; Petry *et al*., 2017; Nawaz *et al*., 2018; Barwell *et al*., 2021; Saltos *et al.*, 2022). Embora *P. capsici* apresente uma ampla gama de hospedeiros, esse patógeno e mais importante nas culturas do tomateiro, em espécies do gênero *Capsicum* e em curcubitáceas do gênero *Cucurbita* (Meitz *et al.*, 2010). Os aspectos

moleculares que condicionam essa plasticidade em termos de colonização de diferentes espécies ainda são desconhecidos. As epidemias são favorecidas por alta umidade relativa do ar e temperaturas em torno de 25ºC (Parada-Rojas *et al.,* 2021).

Na medida em que o agente patogénico é introduzido num campo e suprido por alguma fonte de água (tal como chuva ou irrigação), pode se reproduzir rapidamente por meio da produção de esporângios e zoósporos móveis. Essa rápida e eficiente propagação dentro de um campo pode resultar em perdas de até 100% em alguns dias. A severidade da doença é afetada pelo estado de maturidade da planta. Plantas fenologicamente mais desenvolvidas tendem a se mostrar mais resistentes do que mudas jovens (Saltos *et al.,* 2022). *Phytophthora capsici* é um patógeno que necessita da manutenção de sua planta hospedeira viva até os estágios mais avançados da infecção (McCobe *et al.,* 2022). Jupe *et al*. (2013) observaram que *P. capsici* tem um ciclo de infecção relativamente curto em comparação com organismos relacionados, como *P. infestans*. Nesse ciclo de infecção, a passagem do estágio bitotrófico para necrotófico ocorre 24 horas após inoculação (HAI) e 72 (HAI) ocorre esporulação, com formação de esporângios. A passagem do estágio biotrófico para necrotrófico ocorre mediante ação de efetores do patógeno que desativam o sistema de defesa da planta (Li *et al.,* 2019). Ademais, sugere-se que espécies biotróficas apresentem um círculo mais restrito de plantas hospedeiras. Um exemplo ilustrativo é *P. infestans* que infecta apenas a batata, o tomateiro, e ocasionalmente alguns outros gêneros de Solanaceae. Por sua vez, *P. capsici*, que apresenta a transição do estágio biotrófico para necrotrófico, consegue infectar uma ampla gama de espécies hospedeiras, incluindo plantas de várias famílias botânicas (Cooke *et al.*, 2000).

1.3.3. Taxonomia

Phytophthora capsici pertence ao domínio Eukaryota, reino Straminipila (Sin. Chromista), filo Oomycota, classe Oomycetes, ordem Peronosporales, família Peronosporaceae, gênero *Phytophthora*, espécie *P. capsici* (Leonian, 1922; Mycobank, 2022).

Com o avanço das pesquisas novas espécies foram sendo adicionadas ao gênero *Phytophthora*, confirmando a necessidade de um conjunto de dados multi-locus para identificar com precisão algumas espécies divergentes dentro dos clados (Blair *et al.*, 2018). Atualmente, o gênero *Phytophthora* é composto por ≈ 200 espécies (Brasier *et al*., 2022; Kronmiller *et al.*, 2022). Inicialmente, as relações filogenéticas entre as espécies de *Phytophthora* foram examinadas, principalmente, com base na sequência espaçadora transcrita interna (ITS) do DNA ribossômico genômico (Cooke *et al.*, 2000). O gênero está atualmente dividido em doze clados principais, constituído por um aglomerado monofilético relativamente compacto (Brasier *et al.*, 2022). Os clados filogenéticos compartilham ampla gama de caracteres que caracterizam coletivamente o gênero *Phytophthora* com considerável variação entre as espécies dentro de um clado e entre clados. Os diferentes clados são resultados de deriva genética e adaptação local aos diferentes hospedeiros e partes de hospedeiros, habitats e zonas climáticas nos continentes onde foi relatada (Brasier *et al.*, 2022). Nesse sentido, a hibridação interespecífica é evento comum na evolução do gênero *Phytophthora*, que pode beneficiar a transferência horizontal de genes, sendo esta favorável aos processos de adaptação do patógeno (Richards *et al.*, 2011; Bertier *et al.*, 2013).

É válido ressaltar, que a espécie *P. tropicalis* já foi classificada como *P. capsici*, no entanto análises moleculares usando marcadores AFLP, permitiram diferenciar esta espécie de *P. capsici* (Donahoo & Lamour, 2008). Essas duas espécies apresentam semelhanças morfológicas como esporângios decíduos de forma oblonga com papilas apicais em pedicelos longos e devem cruzar para produzir oósporos anfígenos (Donahoo & Lamour, 2008).

Phytophthora capsici pode ser diagnosticada pela observação da morfologia. Nesse viés, com a ajuda de uma lupa e/ou microscópio é possível identificar os esporângios e observar que estes variam em tamanho (17–39 × 33–66 µm) e forma, sendo papilados e caducos. Os esporângios podem ser subesféricos, ovóides, obovóides, elipsóides, fusiformes

ou piriformes. Os pedicelos variam amplamente em comprimento variando entre 20–260 µm. Cada esporângio pode produzir 20 a 40 oósporos móveis, são comumente produzidos em populações de campo onde ambos os grupos de compatibilidade estão presentes e são o único meio de sobrevivência do patógeno onde as temperaturas são mais baixas no inverno. Em regiões tropicais, as populações de *P. capsici* podem existir por reprodução sexuada ou clonal, caso em que os oósporos podem estar ausentes (Parada-Rojas *et al.,* 2021). No entanto, características morfológicas não são confiáveis para identificar isolados de *P. capsici* (Jayawardena *et al*., 2021; Brasier, *et al.,* 2022). Demonstrando que abordagens moleculares integradas com estudos fenotípicos representam a melhor estratégia para identificação de *P. capsici.*

Diferentes técnicas moleculares já foram usadas para estudar a diversidade genética de *P. capsici*, como: RAPD (Sun *et al*., 2008); AFLP (Guerrero-Aguilar *et al.,* 2022), SNPs (Vogel *et al.,* 2021; 2022), genotipagem de microssatélites usando marcadores SSR (Mohammadbagheri *et al.,* 2021; Fan, *et al.,* 2022) e técnicas de sequenciamento NGS (Lee *et al.,* 2021). Todas essas análises evidenciaram o alto grau de diversidade genética existente entre os isolados de *P. capsici*.

Vários segmentos dos genomas nuclear e mitocondrial têm sido usados para estudos de filogenia e identificação molecular de *P. capsici*. As regiões genômicas mais utilizadas têm sido *cox*1, *cox*2, *nad1*, *nad5*, β-tubulina, EF-1α, Enolase, HSP90, TigA, Ura3 e ITS (Osuna-Avila, 2014; Nawaz *et al.,* 2018; Bhai *et al*., 2022). Esses marcadores foram escolhidos principalmente porque as variações na sequência desses fragmentos de DNA foram eficazes para distinguir espécies intimamente relacionadas. Além disso, esses segmentos genômicos têm sido comumente usados para estudos filogenéticos e taxonômicos de oomicetos. Considerando Lücking *et al.* (2020) os marcadores *cox*1 e *cox*2 seriam aos mais adequados para identificar espécies dentro de Oomycota. Para Crous *et al.* (2021), análises empregando a

combinação de informações das regiões *cox*1, *cox*2, *nad9*, *rps10*, tub2 seriam as mais indicadas para o gênero *Phytophthora*. No entanto, para Chen *et al.* (2022) a nível de gênero seriam as regiões: LSU, ITS, *cox*1 e a nível de espécie: ITS, Btub, TigA, *cox*1. Demonstrando que existe um grupo diverso de marcadores para esse fim, possibilitando diferentes estratégias.

O rDNA evolui de forma relativamente lenta e possui ampla variedade de regiões conservadas e variáveis, o que fornece conveniência para o desenho de *primers* de ampla gama e espécies específicas para detecção molecular de oomicetos. Bhai *et al.* (2022) identificaram que algumas sequências da região ITS de isolados de *Phytophthora* que não correspondiam a nenhuma espécie, indicando o baixo poder de resolução dessa região genômica. Ademais, a região ITS contém grande número de regiões variáveis e, à medida que a distância evolutiva aumenta, a qualidade do alinhamento de múltiplas sequências da região ITS se degrada rapidamente. Desta maneira, a região ITS pode não fornecer uma boa estimativa filogenética para diversos fungos e oomicetos (Bhunjun *et al.*, 2021).

Existem vários estudos de uso de DNA mitocondrial para detecção e quantificação de oomicetos. Assim o gene da subunidade 2 da enzima citocromo oxidase c subunidade II (*cox*2), presente no DNA mitocondrial (**Figura 4**), tem sido a região genômica que demonstra diferenças interespecíficas significativas, sendo um dos mais recomendados para identificação de espécies dentro do gênero *Phytophthora* (Choi *et al*., 2015).

Além disso, as mitocôndrias mantêm um genoma circular e extranuclear, sendo que esse genoma é herdado de forma uniparental, estruturalmente conservado e evoluindo rapidamente no nível da sequência de nucleotídeos. Assim, o genoma mitocondrial tornou-se importante fonte de dados de sequência para resolver relações evolutivas (Winkworth *et al.*, 2022).

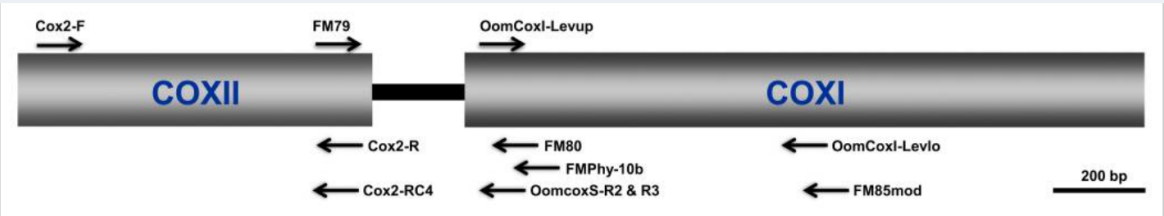

Figura 4. Organização gênica do conjunto de genes mitocondriais cox 2-1 e localização dos primers geralmente usados para PCR. As setas acima ou abaixo do fragmento indicam a direção dos primers usados para amplificação. Fonte: Choi *et al*. (2015).

1.3.4. Grupos de compatibilidade (mating types).

Os isolados de *P. capsici* podem ser subdivididos em grupos de compatibilidade (mating types) que são definidos com base em diferentes aspectos fisiológicos e genéticos que definem aspectos peculiares de comportamento sexual (Uchida & Aragaki, 1980; Vogel, *et al*., 2021). *Phytophthora capsici* é uma espécie heterotálica que se reproduz de forma sexuada somente quando isolados de grupos de compatibilidade distintos estão em contato. Os isolados de qualquer grupo de compatibilidade são hemafroditas e, portanto, capazes de produzir anterídios e oogônios, os órgãos reprodutores, masculino e feminino, respectivamente da fase sexual (Vogel, *et al*., 2021). Cada grupo de compatibilidade secreta um hormônio específico (α 1 ou α 2) que induz a produção de gametângios em isolados do grupo de compatibilidade oposto (Uchida & Aragaki, 1980). É sabido também que um locus com aproximadamente 1,6 Mb controla o grupo de compatibilidade em *P. capsici*, como relatado em Carlson *et al.* (2016).

Como mencionado, em *P. capsici* ambos os grupos de compatibilidade são necessários para que ocorra o cruzamento e a reprodução sexuada. O cruzamento envolve um processo de estimulação que inclui: **(i)** recepção de fatores do tipo acasalamento (estes podem ser ativados através de uma membrana); **(ii)** transição para a produção de gametângios masculinos

(anterídios) e femininos (oogônios) dentro de cada um dos isolados A1 e A2; e **(iii)** crescimento dos anterídios através dos gametângios oogoniais.

A meiose ocorre nos gametângios e os núcleos haploides são transportados para o oogônio através de um tubo de fertilização. Este processo ocorre entre 3–5 dias em condições de laboratório e leva à produção de oósporos (que são esporos de paredes espessas). Os oósporos podem persistir no solo por anos, sendo resistentes a condições ambientais adversas (Babadoost & Pavon, 2013). Os oósporos requerem um período de dormência indeterminado (geralmente acima de 8 semanas) e, dependendo dos parentais, podem produzir progênie sexual viável quando obtidos de cruzamentos efetuados *in vitro*. O cruzamento é relativamente simples, exigindo a co-cultura dos dois grupos de compatibilidade (mating types) A1 e A2 em meio que inclua uma fonte de esteróis (meio ágar de suco V8 = V8 ágar). Os esteróis necessitam ser suplementados no meio uma vez que *P. capsici* (e outros oomicetos) não produz esses compostos que são essenciais para a produção de oósporos viáveis (Lamour *et al*., 2012; Saltos *et al*., 2022; Erwin & Ribeiro, 1996).

Em relação à produção de esporângios de *P. capsici* em meio de cultura, o cultivo em meio V8 a 26ºC tem sido a metodologia com melhor desempenho quanto ao crescimento da colônia, produção de esporângios, oogônios e anterídios (Nawaz *et al.*, 2018). Outrossim, a sobrevivência por longos períodos da fase assexual, pode ocorrer pela formação de clamidósporos. No entanto, a produção de clamidósporos é mais rara em isolados de *P. capsici*. O modo de reprodução varia temporalmente entre as populações em uma dada região. Geralmente, o fluxo gênico entre diferentes áreas é limitado, podendo ocorrer diferentes modos de reprodução nas áreas infestadas (Vogel, *et al*., 2021).

A distribuição geográfica e prevalência dos dois grupos de compatibilidade é muito grande. Pavía *et al.* (2004) relataram no Novo México a presença de ambos os grupos de compatibilidade na mesma região, resultando em reprodução sexual mais frequente. O grupo

A1 foi mais frequente que o A2, no entanto não houve correlação entre grupo de compatibilidade e virulência. No leste dos EUA, foi observado grande diversidade de *P. capsici*. Essa observação foi possível com uso de 12 marcadores microssatélites, com os quais foram identificados cinco grupos genéticos a partir de 157 isolados. Esses cinco grupos estão estruturados por tipo de hospedeiras, com fluxo gênico limitado para alguns estados e menos definidos entre outras áreas. Entre esses isolados, 51% pertencem ao grupo A2 contra 49% do grupo A1. Além disso, a pressão de seleção causada pelo uso de fungicida fluopicolídeo pode influenciar nas estruturas dessas populações (Parada-Rojas *et al.,* 2022). Cinquenta e sete (57) isolados de cucurbitáceas oriundos de diferentes regiões do estado Illinois (EUA) foram avaliados para grupo de compatibilidade, sendo 31 isolados A1 e 26 isolados A2, com a ocorrência de um ou ambos os grupos por região (Islam *et al.,* 2005). Na Geórgia (EUA), 49 isolados foram identificados, dos quais 49% foram identificados como A1 e 51% como A2 (Yin *et al.,* 2012). Foi relatada a presenca dos dois os grupos de compatibilidade em algumas áreas, fato essa sabidamente importante para a recombinação genética e composição da diversidade genética observada nas populações de *P. capsici* da Geórgia (Yin *et al.,* 2012).

Em diferentes regiões do México foi detectada a presença de ambos os grupos de compatibilidade e de isolados homotálicos em menor proporção dado um conjunto de 81 isolados coletados (Castro-Rocha *et al.,* 2016). Populações possivelmente clonais foram observadas ao norte do México com maior frequência que na região central. O alto grau de diversidade genética entre isolados pode ser resultado da reprodução sexual e recombinação genética. Com fluxo gênico limitado, cada região desenvolveu características genéticas peculiares (Castro-Rocha *et al.,* 2016). Estudo feito com 30 isolados de *P. capsici* do estado de Guanajuato no México, observou que cada isolado representava um genótipo único, indicando a ausência de populações clonais. Outrossim, não houve correlação entre

virulência, grupos de compatibilidade ou sensibilidade a metalaxil, bem como com os grupos genéticos identificados por AFLP (Guerrero-Aguilar *et al.*, 2022).

Isolados de *P. capsici* coletados de diferentes hospedeiras em diferentes regiões da costa do Peru, foram identificadas como pertencentes ao grupo de compatibilidade A2. Esses 27 isolados constituíram populações clonais o que foi atribuido as práticas culturais, clima e disponibilidade de hospedeiras ao longo do ano (Hurtado-Gonzales *et al.,* 2008).

Na Argentina (América do Sul), foi observado a exclusiva presença de isolados do grupo de compatibilidade A1. Esse dado foi obtido a partir de 61 isolados de *P. capsici* coletados em 17 campos de produção comercial de hortaliças no Nordeste (NE) da província de Buenos Aires. A ausência do grupo A2 é hipotetizada, como resultado de deriva genética ou ausência de pressão de seleção favorecendo o surgimento do grupo A2, como a disponibilidade de hospedeiras durante todo o ano (Iribarren *et al.*, 2016).

No Brasil (América do Sul), Mchau & Coffey (1995) relataram a presença dos dois grupos de compatibilidade em diferentes hospedeiras, estruturadas em subpopulações. Uma coleção contendo 130 isolados foi avaliada por Paz-Lima (2006) tendo sido relatado os dois grupos de compatibilidade por hospedeira, dentro dos estados brasileiros de Goiás, São Paulo, Minas Gerais, Santa Catarina, Pernambuco, Bahia, Paraíba e Distrito Federal. A identificação *in vitro* de 130 isolados de vários estados do Brasil por Paz-Lima (2006) observou que 87% desses isolados pertenciam ao grupo A1. Outrossim, foi evidenciado que os isolados do grupo A1 causaram mais doença que isolados do grupo A2. Esse fato também foi relatado por Barchenger *et al*. (2017) em que isolados classificados como A1 (diploides) causaram mais doença que isolados A2 (triploides).

Supreendentemente, a ploidia pode estar relacionada a determinação do grupo de compatibilidade. De fato, a ploidia em *P. capsici* pode ser variável, o que destaca uma nova forma de plasticidade dentro do genoma deste patógeno, sendo observada, inclusive, a

existência de aneuploides, de origem mitótica e poliploides de origem meiótica (Vogel, *et al.*, 2021). Nesse sentido, os isolados predominantemente triploides são, em geral, menos patogênicos do que os isolados diploides (Barchenger *et al.,* 2017). Por outro lado, as linhagens clonais mais bem-sucedidas em *P. infestans* são triploides. No entanto, um genótipo triploide é capaz de retornar rapidamente a um estado diploide que pode ser mais adequado para reprodução sexual com outros isolados com constituição genética 2n (Li *et al.,* 2017).

Em suma, compreender e catalogar a variabilidade e a dinâmica de populações naturais de *P. capsici* é de extrema importância para desenvolver estratégias de delimitação de espécies e para o estabelecimento de estratégias de manejo de doenças ecologicamente corretas e eficazes (Hyd *et al.,* 2020).

1.4. Melhoramento do tomateiro para resistência ao oomiceto *Phytophthora capsici*: Recursos genéticos e genômicos disponíveis

Um aspecto fundamental para os programas de melhoramento visando resistência genética a doenças é o conhecimento da variabilidade do patógeno bem como a identificação do maior número possível de fontes de resistência. Para que um programa de melhoramento genético visando resistência seja bem sucecedido se faz necessário conhecer a amplitude e o tipo de herança em cada fonte de resistência detectada. Essas informações vão definir a escolha da estratégia mais eficaz e adequada para o desenvolvimento de novas cultivares. De acordo com Petry *et al*. (2017), um número maior de acessos deve ser avaliado em virtude da baixa frequência relativa fontes com altos níveis de resistência ao oomiceto *P. capsici* detectadas em avaliações iniciais do germoplasma de *Solanum* (*Lycopersicon*). Detectar novas fontes de amplo espectro bem como revalidar as fontes de resistência já disponíveis com diferentes isolados de *P. capsici* são ações de pesquisa importantes a fim de confirmar a estabilidade desses acessos contra um patógeno com ampla diversidade. De fato, os resultados de manejo têm indicado que identificar, conhecer e manipular genes que determinam

resistência ou tolerância a esse patógeno parece ser a única maneira de maximizar o rendimento das culturas agrícolas hospedeiras (Garrido-Cardenas *et al.,* 2018). No que se refere a resistência de plantas a doenças, o uso da seleção assistida por marcadaores (SAM) tem se mostrado como uma ferramenta com excelentes resultados, melhorando a eficiência e precisão dos programas de melhoramento do tomateiro (Fonseca & Boiteux, 2021).

Uma das maneiras clássicas de avaliar o controle genético de uma característica em plantas é a condução de estudos de herança com cruzamentos controlados entre parentais contrastantes (cruzamento biparentais). O estudo de herança é feito a partir da obtenção de populações segregantes para a característica alvo, e avaliação das proporções fenotípicas observadas. Assim podem ser obtidas as gerações F_1, F_2, (podendo-se inserir demais gerações avançadas) e os retrocruzamentos (RC para o parental resistente e RC para o parental suscetível). Posteriormente, todas as populações obtidas, incluindo os genitores, são avaliadas (inoculadas) para o caráter de interesse para verificar a segregação fenotípica. Como mencionado, deve-se realizar inicialmente o cruzamento entre duas variedades homozigotas contrastantes para a característica de interesse. As sementes resultantes constituem a geração F_1. As plantas F_1 devem ser autopolinizadas (= autofecundadas), para obtenção das sementes F_2. Nas plantas da geração F_2, avalia-se a característica de interesse em um grande número de indivíduos.

A população F_2 também tem sido a geração mais empregada em estudos de mapeamento genético de genes de resistência (Fonseca & Boiteux, 2021). As plantas F_2 devem ser desafiadas com o agente causal da doença em estudo, anotando-se o número de plantas resistentes e suscetíveis. As plantas F_1 são retrocruzadas (geração BC do inglês *back-cross*) com os parentais a fim de serem avaliadas para a característica de interesse, obtendo-se assim o número de progênies que são 100% resistentes, 100% suscetíveis e as que estão segregando, ou seja, que apresentam tanto plantas resistentes quanto suscetíveis. Os dados

obtidos nas avaliações nas gerações F_2 e de retrocruzamento são comparados com os dados esperados sob uma determinada hipótese genética. Para caracteres com distribuição discreta, as hipóteses de segregação são avaliadas pelo teste de qui-quadrado, que verifica se os desvios entre as frequências observadas e esperadas são significativos, em determinado nível de probabilidade.

A domesticação do tomate é distinta da diferenciação das espécies pela seleção natural, como consequência da seleção de um conjunto limitado de características, incluindo a forma e o tamanho do fruto. Assim, sua base genética do tomateiro cultivado foi reduzida, dificultando o melhoramento do mesmo para caracteres como resistência (Bauchet & Causse, 2012). A fim de ampliar a base genética, os programas de melhoramento se concentram na introgressão de genes de interesse oriundos de espécies silvestres em linhagens elite, o que pode ser problemático, devido os mecanismos de autoincompatibilidade.

Como o primeiro passo da hibridação introgressiva envolve cruzamentos do tomate cultivado com espécie contrastante, podendo ser espécie silvestre ou espécies mais distantes do clado de tomate. É importante ter o máximo de informação sobre os parentais contrastantes para aumentar a eficiência do programa de melhoramento (Anisa *et al.*, 2022).

Em termos de detecção de marcadores moleculares para serem empregados em processos de seleção assistida, a disponibilidade da sequência do genoma do tomate possibilitou realizar diversos estudos em Solanaceae (Fonseca & Boiteux, 2021). O genoma de referência do tomate 'Heinz 1706' é um recurso que pode ser usado para explorar e catalogar a variabilidade e diversidade existentes, intraespecífica e interespecífica, a fim de monitorar via marcadores moleculares importantes características agronômicas, como resistência às principais doenças, no tomate cultivado. Os genomas obtidos via Next-Generation Sequencing (**NGS**) ou High-Throughput Sequencing (**HTS**) permitiram ancorar fisicamente todo o repertório de genes de uma espécie, auxiliando em estudos de associação

de fenótipo-genótipo, na compreensão da expressão de genes, interação proteína-DNA, identificação de mutações, análise do proteoma, epigenoma, acelerando a descoberta de novos mecanismos regulatórios do tomateiro. Além disso, ajudou no processo de conhecimento dos mecanismos evolutivos conservados ou não conservados (Kumar & Khurana, 2014).

1.4.2. Identificação de fontes de resistência genética no tomateiro ao oomiceto *Phytophthora capsici*

O tomateiro é uma das hortaliças mais importantes no mundo, sendo consumido de diversas formas, em diferentes regiões (Foolad, 2007). A podridão basal, causada por *P. capsici* foi descoberta pela primeira vez no Novo México, infectando *Capsicum annuum* (Leonian, 1922). *Phytophthora capsici*, causa a podridão das raízes, bem como a podridão do caule, das folhas e dos frutos. Esse patógeno já foi relatado infectando diversas hospedeiras e tem sido amplamente disseminado por diversos países tornando-se motivo de preocupação, uma vez que o manejo se torna difícil após o seu ingresso em determinada área (Pontes *et al.*, 2014; Barboza *et al*., 2017; Petry *et al*., 2017; Nawaz *et al*., 2018; Barwell *et al*., 2021; Saltos *et al.*, 2022). Uma vez introduzido em novos campos de produção e tendo a disponibilidade de alguma fonte de água livre no solo (exemplo chuva ou irrigação), esse patógeno pode se reproduzir e se disseminar rapidamente por meio de esporângios e zoósporos móveis. Essa rápida e eficiente propagação dentro de um campo pode resultar em perdas de até 100% dentro de um período curto de tempo. A severidade da doença é afetada pelo estado de maturidade da planta, sendo que plantas fenologicamente mais desenvolvidas tendem a se mostrar menos suscetíveis do que mudas ou frutos jovens. Isolados de *P. capsici* podem se reproduzir tanto assexuadamente quanto sexualmente (Saltos *et al.*, 2022).

Os sintomas variam consideravelmente de acordo com a hospedeira, parte da planta infectada e condições ambientais (Granke *et al*., 2012). Em áreas secas a infecção em tomateiro e em espécies de *Capsicum* é geralmente observada nas raízes. Além disso, plantas

infectadas têm uma lesão distinta, preta/marrom visível no colo (**Figura 2**). Em áreas em que as chuvas são mais frequentes, todas as partes da planta podem apresentar sintomas, incluindo as raízes, parte aérea, folhas e frutos. Infecções radiculares causam tombamento das plântulas, ao passo que, em plantas mais velhas, é comum observar desenvolvimento atrofiado, murcha e, eventualmente, morte. No tomateiro, é comum observar um crescimento significativo de raízes adventícias, com plantas atrofiadas, embora severamente comprometidas, podem não morrer, no entanto tem sua produtividade reduzida.

Geralmente, as estratégias de controle têm por objetivo limitar as perdas associadas com o agente patogénico. No caso particular de *P. capsici*, quando esse patógeno se estabelece em nova região de cultivo pode ser muito difícil implementar estratégias eficientes de controle. Essas abordagens de manejo incluem irrigação adequada, rotação de culturas, solarização do solo, aplicações de fungicidas e o plantio de cultivares resistentes aos isolados locais.

A resistência a *P. capsici* em várias plantas hospedeiras é influenciada por vários fatores, incluindo fatores ambientais, perfil de virulência dos isolados (raças fisiológicas ou patotipos) e à fonte de resistência. Isolados de *P. capsici*, devido ao curto ciclo de vida e rápida evolução de virulência, têm vantagem seletiva sobre seus hospedeiros que apresentam evolução mais lenta na interação planta-patógeno (Lee *et al.,* 2021). Como mencionado, a virulência de isolados de *P. capsici* é outro fator que pode afetar a análise genética dos mecanismos de resistência. A presença de isolados capazes de induzir menores níveis de severidade em dada planta hospedeira pode levar a detecção de "falsa resistência", ao passo que isolados capazes de induzir danos severos podem suplantar a resistência de alguns acessos de plantas hospedeiras (Grünwald *et al.,* 2017; Barchenger *et al.,* 2018; Retes-Manjarrez *et al.,* 2020).

Neste cenário, o melhoramento genético para resistência ao oomiceto *P. capsici* é um processo complexo. Em *Capsicum*, por exemplo, os níveis de resistência obtidos em

cultivares comerciais não são tão elevados aos das fontes de resistência originais (Barchenger *et al.,* 2018). Mesmo assim, o melhoramento genético para resistência durável ao *P. capsici* ainda é um objetivo importante da maioria dos programas de melhoramento (Karasov *et al.,* 2020). Além disso, a diversidade no perfil de virulência de isolados de *P. capsici* e o complexo mecanismo genético em algumas fontes de resistência nas plantas hospedeiras são os principais fatores limitantes no melhoramento de resistência a *P. capsici* (Barchenger et al., 2018; Retes-Manjarrez *et al.,* 2020).

Diferentes padrões de herança têm sido propostos na resistência à *P. capsici* em *Solanum*: Quesada-Ocampo *et al.* (2010) identificaram o acesso *Solanum habrochaites* 'LA 407', como resistente a quatro isolados de *P. capsici*. Ademais, marcadores AFLP demonstraram que os genótipos de tomateiro avaliados não apresentaram correlação entre agrupamentos genéticos e suscetibilidade à *P. capsici*, indicando que a resistência está muito provavelmente distribuída em diferentes linhagens de tomateiro. Um estudo posterior de Quesada-Ocampo *et al*. (2016) forneceu evidências indiretas em relação à potencial existência de raças fisiológicas/patotipos no patossistema *P. capsici*–tomateiro uma vez foi detectada variação fenotípica nos níveis de resistência para cada linhagem relacionada com a virulência dos isolados. Quesada-Ocampo *et al*. (2016) também forneceram evidências sobre a natureza quantitativa bem como de respostas do tipo isolado-específicas de linhagens de tomateiro à infecção por *P. capsici.* Estes dados sugerem que a resistência à *P. capsici* nesse acesso de tomateiro é muito provavelmente poligênica, situação similar com aquela observada em *Capsicum* que também apresenta uma herança complexa.

Resistência a uma ampla gama de isolados tem sido relatada exclusivamente no acesso *C. annuum* 'Criollo de Morelos 334' (CM334). Lozada *et al*. (2021) identificaram três regiões genômicas de controle quantitativo (QTL.Pc5, QTL.Pc8.1 e QTLPc.9) nos cromossomos 5, 8 e 9 após avaliação de linhagens RIL (*recombinant inbred lines*), originárias

da hibridação entre ‘CM-334’ e a cultivar suscetível ‘Early Jalapeno’. Foram observadas interações aditivas entre os QTLs dos cromossomos 5 e 8 para resistência à *P. capsici*. Além disso, identificaram o cromossomo 5 como sendo um ‘*hotspot’* genético envolvido em múltiplas e diversas funções genéticas associadas a diferentes processos biológicos, incluindo resposta de defesa, reparo de DNA, regulação da atividade da quinase e mecanismos epigenéticos, como metilação do DNA e remodelação da cromatina.

Para Kim *et al.* (2019) a resistência à *P. capsici* em *Capsicum* é controlada por muitos loci (QTLs), sendo o QTL do cromossomo 5 um dos principais fatores de resistência. Nesse estudo, foram utilizados 11 marcadores baseados em genes de resistência ortólogos (RGA) que estão ligados aos principais QTLs para resistência à *P. capsici*. Entre os marcadores, CaNB-5480 mostrou-se o mais intimamente ligado ao principal QTL. A combinação dos marcadores CaNB-5480, CaRP-5130 e CaNB-5330 geraram o sistema de genotipagem mais preciso para detecção de resistência contra *P. capsici* de acordo com a validação usando 61 acessos de *Capsicum.* Em estudo conduzido por Siddique *et al*. (2019), dois QTLs para resistência do tipo isolado-específica (QTL5.1 e QTL5.3) e um QTL de amplo espectro (QTL5.2) foram detectados no cromossomo P5 em bioensaios empregando três isolados de *P. capsici*. As eventuais disparidades observadas nos resultados de diferentes grupos de pesquisa podem ser ocasionadas pelo uso de diferentes parentais contrastantes, diferentes isolados, densidades de inóculo, métodos de inoculação, tempos de avaliação e/ou condições ambientais.

Uma abordagem investigada mais recentemente é a descrição e catalogação dos efetores do patógeno a fim de identificar novos genes R na planta (Dangl *et al.,* 2013). Um estudo de Li *et al.* (2019) caracterizou o efetor RxLR207, associado à passagem do estágio biotrófico para necrotrófico de *P. capsici* usando *Arabidopsis* como modelo. Esse efetor serviu de sonda para identificação do gene BPA1, codificado dentro de um “cluster” de genes conservados,

que estão relacionados ao sistema de defesa da planta, mais precisamente à ação das espécies reativas de oxigênio (ROS). O grupo de efetores RXLR é conhecido como fatores de avirulência (Avr) contra o hospedeiro resistente com genes R associados (Arif *et al.*, 2021).

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CAPÍTULO II

Phytophthora capsici isolates infecting Solanaceae and Cucurbitaceae in Brazil: Geographic and host prevalence of mating types and *cytochrome-c oxidase subunit* 2 (*cox*2) gene haplotypes.

Phytophthora capsici **isolates infecting Solanaceae and Cucurbitaceae in Brazil: Geographic and host prevalence of mating types and *cytochrome-c oxidase subunit* 2 (*cox*2) gene haplotypes.**

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Abstract

Phytophthora capsici Leonian (Peronosporales, Pythiaceae) is a pathogen of broad adaptability, inducing severe losses in multiple hosts in Neotropical regions. However, the knowledge about the genetic and phenotypic diversity of isolates affecting Solanaceae and Cucurbitaceae crops is yet incipient in Brazil. Here, we carried out characterization studies of *P. capsici* isolates via: **(1)** phylogenetic analysis and haplotype determination employing the mitochondrial *cytochrome-c oxidase subunit* 2 (*cox*2) gene; **(2)** determination of geographic and host incidence of the mating types (MTs) by using *in vitro* assays and by employing a molecular marker system and **(3)** pathogenicity bioassays. PCR amplification of the *cox*2 produced amplicons of 850 bp for all 45 isolates. A subset of 18 isolates was selected for phylogenetic inference. The usefulness of *cox*2 gene was confirmed for distinguishing *P. capsici* from closely related taxa. Eighteen isolates were placed into six haplotypes. In pathogenicity tests, a subgroup of *P. capsici* isolates capable of inducing symptoms in *Capsicum* fruits was avirulent in tomato seedlings. Tweenty-one out of 45 isolates were MT A1 and 24 were A2. MT switches were observed in both directions. The simultaneous presence of both MTs was detected in the same production area and in all major hosts. Alternative molecular techniques should be developed for MT determination of Brazilian isolates, since the available marker system displayed inconsistent results with our *in vitro* assays. Our characterization expands our knowledge of *P. capsici* diversity and represents important pieces of information towards the sustainable management of this pathogen in Neotropical areas.

Keywords: Phylogeny, molecular markers, diversity, *cox*2 gene.

Introduction

The oomycete *Phytophthora capsici* Leonian (Peronosporales, Pythiaceae) is a pathogen with wide adaptability, causing total yield losses in multiple hosts, especially within the families Solanaceae and Cucurbitaceae in tropical and subtropical regions (Leonian 1922; Yin *et al.* 2012; Gobena *et al.* 2012; Reis *et al.* 2018; Brasier *et al*. 2022). *Phytophthora capsici* can induce a complex of symptoms that vary according to the host, infected tissue, stage of plant development, and environmental conditions (Barchenger *et al*. 2018a; Parada-Rojas *et al.* 2021). The diseases caused by this pathogen can manifest as stem rot and basal browning, root and fruit rot, wilting, plant blight, and even complete plant collapse (Barchenger *et al.* 2018b; Parada-Rojas *et al.* 2021).

The family Peronosporaceae (Oomycota, Chromista) is a monophyletic group of biotrophic and hemibiotrophic plant pathogens (Thines and Choi 2016), comprising around 1,000 species with 200 of them placed within the genus *Phytophthora* (Thines and Choi 2016; Winkworth *et al*. 2022). Currently, the Peronosporaceae family comprises 19 genomic clades (Winkworth *et al*. 2022). Species belonging to 15 of these clades have a fully characterized mitochondrial genome (Winkworth *et al.* 2022). The mitochondrial genome contains introns and encodes several essential eukaryotic functions linked to energy production, virulence, and pathogenicity. The small size of the mitochondrial genome has facilitated the characterization of several fungal and oomycete genomes (= mitogenomes). A search for mitogenomes in the GenBank (January 15, 2023) showed a total of 16,504 available genomes, 55 of them from *Phytophthora* species. Mitogenomes have, in general, 14 genes that code for an array of functions including: **(1)** oxidative phosphorylation and ATP production: *atp6*, *atp8*, *atp9* (ATP synthase subunits), *cob* (cytochrome b), *cox*1, *cox*2, and *cox3* (subunits of cytochorme oxidase), *nad*1, *nad2*, *nad3*, *nad4*, *nad5*, and *nad6* (subunits of NADH dehydrogenase); **(2)**

genes that encode rRNAs: *rns* and *rnl* and an *rps3* gene responsible for translation and composition of mitochondrial ribosome units; and **(3)** tRNA encoding genes (Kulik *et al.* 2020; Winkworth *et al.* 2022).

Even though the identification of the genus *Phytophthora* using morphological characteristics is considered stable and reliable (Granke *et al.* 2011; Parada-Rojas *et al.* 2021), molecular techniques are necessary for a more accurate diagnosis at the species level. Different genomic regions have been used for species identification, including mitochondrial and nuclear genes/genomic regions. The genetic markers largely employed in these studies are the cytochrome-*c* oxidase 1 (*cox*1), the 5.8S ribosomal DNA gene and internal transcribed spacers (ITS rDNA); 60S ribosomal protein L10, beta-tubulin (β-*tub*), elongation factor 1-alpha, enolase, heat shock protein 90, 28S ribosomal DNA, and the *tig*A gene fusion protein (Quesada-Ocampo et al. 2011; Guha and Grünwald, 2012; Martin *et al*. 2014; Yang and Hong, 2018; Rai *et al.* 2020; Jung et al., 2022). The genus *Phytophytora* is composed of 12 major clades in a monophyletic cluster according to multilocus analyses using information from the internal transcribed spacer region (ITS rDNA), β-tubulin, *cox*1, and *nadh*1 regions (Brasier *et al*. 2022). The employment of these nuclear markers in combination with mitochondrial markers in a multilocus phylogeny of 82 *Phythophthora* species confirmed the presence of ten clades with high support, allocating *P. capsici* in clade 2 together with isolates of *P. tropicalis* and *P. mexicana* (Cooke *et al*. 2000; Blair *et al.* 2008; Lamour *et al.* 2012b). The ITS region displays limitations for discrimination of *P. capsici* due to insufficient variability to distinguish phylogenetically very close species (Kulik *et al.* 2020). In fact, clade 2 is one of the most difficult to define within the genus. *Phytophthora tropicalis* includes a subgroup of isolates formerly classified as *P. capsici* as well as isolates reclassified as *P. palmivora* (Aragaki and Uchida, 2001).

The evolutionary history of the mitochondrial *cox*2 gene has been also widely used for identification, taxonomy, and phylogenetic relationships of members of the Peronosporaceae family (Hudspeth *et al*. 2000; Martin *et al*. 2014; Choi *et al.* 2015). The gene *cox*2 was compared with *cox*1 for the identification of several oomycetes (Choi *et al.* 2015). The results indicated that *cox*2 was more efficient in identifying species displaying greater interspecific divergences, being suggested as a barcode gene along with ITS rDNA region (Choi *et al.* 2015).

Approximately half of the *Phytophthora* species are homothallic/self-fertile (Erwin and Ribeiro, 1996). For homothallic species, a single isolate can complete the sexual cycle and producing oospores, which are structures with thick, resistant walls that survive in the soil for long periods (Parada-Rojas *et al.* 2021). The other species are heterothallic and they require the presence of isolates from two distinct mating types for the crossing to occur (Erwin and Ribeiro, 1996). *Phytophthora capsici* is heterothallic, presenting both asexual and sexual reproduction systems (Erwin and Ribeiro, 1996). This trait is associated with to the continuous emergence of subpopulations of this pathogen with remarkable variability (Hu et al. 2020). The presence of numerous physiological races/pathotypes in isolates that infect *Capsicum* has been attributed to distinct mechanisms, including genetic recombination via sexual reproduction, mutations, loss of heterozygosity, intragenomic, and intergenomic ploidy variation (Lamour *et al.* 2012a; Ribeiro and Bosland, 2012; Barchenger *et al.* 2017; Barchenger *et al.* 2018b). However, the phenotypic plasticity observed in this pathogen is more likely acquired via increased frequency of a given advantageous allele (match or resistance group) not by meiotic recombination. Simple sporulation and/or growth via mitosis with changes in the copy number of some chromosomes and loss of heterozygosity have been suggested as an explanation for these levels of variation (Hu *et al.* 2020). The phenomenon of

loss of heterozygosity does not present Mendelian inheritance and had already been previously reported after the analysis of the first sequenced genomes (Lamour *et al.* 2012a). Genome re-sequencing of single zoospore isolates revealed a yet elusive phenomenon named as Dynamic Extreme Aneuploidy (DEA), which was associated with the genetic diversity of *P. capsici* (Hu *et al.* 2020). DEA is characterized by the asexual inheritance of diverse intra-genomic combinations of chromosomal ploidy ranging from 2N to 3N and heterozygous allele frequencies that do not strictly correspond to the overall ploidy level. DEA can explain the rapid increase of advantageous alleles and copy neutral loss of heterozygosity in *P. capsici* (Hu *et al.* 2020). Fluctuations in the ploidy level may be a key factor in the adaptation process of *P. infestans* as well (Li *et al.* 2017b).

The opposite mating types of heterothallic *Phytophthora* species secrete specific hormones (either α1 or α2) that induce sexual reproduction in isolates from different mating types (Ko, 1978). Genetic studies indicate that a single locus controls this phenotype in *P. capsici* (Lamour *et al.* 2012b; Carlson *et al.* 2017) and in *P. infestans* and *P. parasitica* (Fabritius and Judelson 1997; Li *et al.* 2017b). However, the molecular identity of the factor(s) that control this trait remain elusive in oomycetes (Vogel *et al.* 2020). The simultaneous presence of *P. capsici* isolates belonging to different mating types (A1 and A2) has been registered in different production fields, opening the possibility of emergence of new variants of the pathogen via recombination (meiosis) between isolates of the two mating types (Lamour and Hausbeck, 2000; Sanogo *et al.* 2022). A PCR-based assay for distinguishing between A1 and A2 mating types based on microsatellites was developed for characterization of Chinese isolates of *P. capsici* (Li *et al.* 2017a). This molecular marker was cloned and converted into a detection system via PCR with specific primers that amplified a 997-bp fragment present

exclusively in the A1 isolates. However, since then, no additional studies were carried out to validate these molecular markers using isolates from other continents.

Previous studies have indicated that the switch of A2 isolates to A1 occurs with higher frequency (Hu *et al.* 2020). However, a more recente and extensive characterization of over 600 field isolates of *P. capsici* from US, South America, Europe and China indicated an opposite trend (Hu *et al*. 2020). In these long-term preserved isolates (around 10 years), A2 mating type was highly unstable, whereas the A1 mating type was stable. DEA was associated with mating type switches observed in this colletion of isolates (Hu *et al.* 2020).

From the point of view of pathogen management and genetic improvement of host species, knowledge of the diversity of *P. capsici* populations makes it possible to monitor disease outbreaks, emergence of resistance to fungicides as well as novel pathotypes able to overcome sources of resistance. However, cataloging the genetic and phenotypic diversity of Brazilian *P. capsici* isolates is yet incipient. In this context, the objective of the present work expands the analysis of the variability of *P. capsici* isolates obtained from different hosts and regions of Brazil via: **(1)** phylogenetic analysis and haplotype determination, using sequence information from a region of the mitochondrial gene *cytochrome oxidase 2* (*cox*2); **(2)** geographic and host distribution of mating types, determined via *in vitro* isolate pairing assays and via molecular markers of Li *et al.* (2017a) and **(3)** pathogenicity and virulence profile bioassays.

Material and Methods

***Phytophthora capsici* isolates –** A collection of 45 *P. capsici* and *P. capsici*-like isolates belonging to the Fungi and Oomycetes Bank at the Phytopathology Laboratory of Embrapa Hortaliças, Brasília–DF, Brazil was used in the present study (**Table 1**). These isolates were

obtained from different hosts in different Brazilian states and the Federal District. Initially, the isolates were recovered from test tubes with V8 medium, preserved in mineral oil. Then the isolates were stored under room temperature conditions in Petri dishes with selective V8 medium. After this step, all isolates were purified via monosporic cultures to ensure genetic uniformity.

DNA extraction of *Phytophthora capsici* isolates and PCR assays of the *cox*2 gene – Total DNA was extracted from all 45 isolates using a modified CTAB methodology and organic solvents (Boiteux *et al*. 1999). Quantity and quality of DNA samples were estimated by absorbance readings using Nanodrop® (Thermo Fisher). This DNA was resuspended in milliQ water (20 ng/µL) and used as a template in PCR assays. The primer pair **PF34** (5'–GGC AAA TGG GTT TTC AAG ATC C–3') and **PF35** (5'–CCA TGA TTA ATA CCA CAA ATT TCA CTA–3') was employed to amplify the *cox*2 mitochondrial region (Hudspeth *et al*. 2000). PCR assays were performed in a total volume of 25 µL, containing 30 ng of DNA, 1X buffer, 2 mM $MgCl_2$, 1 µM of each dNTP, 12 µM of each primer, 0.25U of recombinant *Taq* polymerase (Invitrogen) in milliQ water. The conditions used for amplification were 4 minutes of denaturation at 96°C, followed by 35 cycles at 96°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute with a final extension step at 72°C for 4 minutes in a thermocycler. The size of the amplicons was estimated by analysis on a 1% agarose gel. The amplicons were purified according to the protocol of the Wizard SV Gel and PCR Clean-Up System kit® (Promega) and sequenced by the Sanger dideoxy method in both directions in an ABI 3100 sequencer from Universidade Catolica de Brasilia.

Alignment and analysis of *cox*2 sequences of *Phytophthora capsici* isolates – A subset of 19 isolates with high sequence quality (i.e. well-defined nucleotides and quality above 50), according to Seqman software (Lasergene®, Madison, WI), was selected for molecular

analyses. Data from the *cox*2 gene of *Phytophthora* species used for comparison with the isolates in this work were obtained from reference mitochondrial genomes deposited in GenBank. (https://www.ncbi.nlm.nih.gov/data-hub/genome/?taxon=763924). The contigs of each isolate were constructed by combining the sequences obtained with the two primers (PF34 and PF35) in the SeqMan software (Lasergene®, Madison, WI). The sequences of the isolates were aligned using the Geneious® 8.1.9 program using the ClustalW algorithm. The contigs were manually adjusted to ensure that the codon alignments were maintained according to the reference sequences. Phylogenetic analyzes were performed using Maximum Likelihood (ML) and Bayesian inference (BI). The two methods available as "plugins" of the Geneious® 8.1.9 program were used in this work. For ML the parameters used were: 1000 pseudoreplicates (−m GTRGAMMA -p 12345 -k -f a -N 1000 -× 12,345), GTR GAMMA model. For BI, initially the best evolutionary replacement model was estimated by the MrModeltest 2.3 program (included in the PAUP plugin), being defined as HKY85. Four "Markov Chain Monte Carlo" (MCMC) algorithm were conducted for 10^7 generations, with samplings every 1,000 generations. The convergence of all the parameters was checked using Tracer version 1.5 (Rambaut and Drummond 2010) and the first 25% generations were discarded as burn-in. The program FigTree version 1.4.3 (Rambaut, 2012) was used to visualize the trees, which were edited in Adobe Illustrator CS6 software (Adobe Systems, USA). The majority of the clades displayed ML bootstrap support values above 50 and Bayesian posterior Probability values above 0.50.

Definition of haplotypes of the *cox*2 gene of *Phytophthora capsici* isolates – The number of haplotypes from the *cox*2 gene of the *Phytophthora* isolates was calculated using DnaSP v.5 (Librado and Rozas, 2009). A haplotype network was reconstructed with PopART (Leigh and

Bryant, 2015) using the TCS algorithm (Clement *et al*., 2000, Clement *et al*., 2002) with exclusion of gaps.

Determination of mating types of *Phytophthora capsici* isolates via colony pairing – The isolate PCp-120 was used as a reference for the A1 and the isolate PCp-192 for the A2. The mating type determination assasys were carried out essentially as previously described (Kaosiri and Zentmyer, 1980). The pairing of the known mating type A1 and A2 was done in 20% clarified V8 juice agar plates at 20–25°C by placing the isolates 30 mm apart from each other. The 45 isolates were paired with the two reference isolates. As controls, pairings were made between the known isolates A1 and A1, A2 and A2; and A1 with A2. The Petri dishes were sealed with plastic film and incubated in the dark at 26 °C for 15 days. In the evaluation, slides were prepared from material taken from the intersection of the two colonies and examined under microscope. The presence of oogonia and antheridia indicated that the tested isolate was of the opposite mating type of the reference isolate.

Validation of a methodology for mating type determination via PCR employing Brazilian isolates of *Phytophthora capsici* – The DNA of each isolate was used as a template in PCR assays with primers **Pcap1** (5'–ACG AGT ACG AGT GCT TGG T–3') and **Pcap2** (5'–TGA GTC TCG AGA CAG AGA G–3'), which is able to amplify a specific amplicon (997 bp) for matying type A1 and a specific amplicon of 500 bp for A2 isolates (Li *et al.*, 2017a). PCR assays were conducted in a total volume of 12.5 µL, containing 40 ng of DNA, 1X buffer, 40 mM $MgCl_2$, 1 µM of each dNTP, 5 µM of each primer, 0.2U of *Taq* polymerase (Invitrogen) in milliQ water. The PCR conditions were as follow: 5 minutes of denaturation at 94 °C, followed by 30 cycles at 94°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 1 minute; with final extension at 72ºC for 10 minutes; in thermocycler. PCR products were analyzed on a 1.5% agarose gel.

Characterization of aggressiveness of *Phytophthora capsici* isolates in *Capsicum annuum* fruits – Ten isolates were arbitrarily selected for this test viz. PCt-40, PCt-27, PCt-26, PCa-29, PCa-31, PCp-183, PCp-167, PCVagem, PCpe-09 and PCa-09 representing different hosts and regions. Each isolate was inoculated on five bell-pepper fruits (i.e. five replications per isolate) wounded in two opposite sides with a toothpick. Mycelial discs (1 cm diameter) were then deposited in these wounds. Four days after inoculation, the diameter of the lesion was measured in two opposite directions. Mean lesion diameter data were submitted to ANOVA and the 5% Scott-Knott test in R package (R Core Team 2022; https://www.R-project.org/).

Pathogenicity test of *Phytophthora capsici* isolates on tomato and bell-pepper seedlings – The isolates used in the aggressiveness test on bell-pepper fruits were also employed in controlled inoculation bioassays with seedlings of tomato cultivar 'Santa Clara' and bell-pepper cutivar 'Tico'. For inoculum preparation, pure cultures were transferred to Petri dishes containing 20% V8 agar medium (100 mL V8 juice, 1.5 g calcium carbonate, 10 g of agar, and 400 mL distilled water). Cultures were maintained in growth chambers for 3 days in the dark and for 3 to 5 days under continuous light (25 ± 2 °C) to induce abundant sporangia production. Then, 20 mL of sterile distilled water was added to each plate and the plates were transferred to 6 °C for one hour, followed by 30 minutes at 25 °C to induce zoospore production. The zoospore suspension was homogenized and filtered through sterile gauze. The estimate of the zoospore concentration was made with the aid of the Neubauer chamber. Tomato and bell-pepper seedlings were grown in styrofoam trays with 128 cells. When the young seedlings had two pairs of fully opened true leaves (25 days after sowing), they were removed from the tray cells and transplanted into 1.5 L plastic pots (three plants per pot) containing sterile substrate. Seedlings were then inoculated via deposition of 3 mL around the crown area of a suspension adjusted to 2×10^4 zoospores mL^{-1} for each isolate (separately).

Results

Phylogenetic analysis of *Phytophthora capsici* isolates using the mitochondrial *cox*2 gene sequences – PCR amplification of the *P. capsici cox*2 region produced PCR fragments of approximately 850 bp for all 45 isolates (**Figure 1**). The sequences of the subset of 18 isolates were aligned using Seqman software (Lasergene®, Madison, WI) and they displayed identity levels ranging from 98 to 99% among them. The alignment initiated at position 98 (98-100 = codon GGT-Glicyne) of the COX 2 protein (**Figure 2**). Evolutionary reconstruction using maximum likelihood and Bayesian inference allowed the placement of 18 isolates into the *P. capsici* clade 2 (**Figure 3**). All taxa were supported by high bootstrap values and posterior probabilities.

Haplotypes of *Phytophthora capsici* isolates using the *cox*2 gene sequences – Low intraspecific variation was observed in *cox2* sequence data for the 19 *P. capsici* isolates with the identification of seven haplotypes. The haplotype 1 was composed of 11 isolates from different hosts, geographic regions, and of different mating types. The haplotype 2 was composed of three isolates (PCt-38, PCa-01, and PCa-31), whereas the haplotype 3 was represented by the PCC-004 isolate. The isolate PCC-04 displayed an atypical clustering pattern closer to the *P. nicotianae* clade (**Figure 4**). The haplotype 4 was composed of the PCp-115 isolate; haplotype 5 by the isolate PCa-09; haplotype 6 by the isolate PCp-192, and haplotype 7 by the isolate PCt-26 (**Figure 4**). No correlation was observed of haplotypes and hosts, collection areas, mating types or aggressiveness.

Determination of mating types of *Phytophthora capsici* isolates – The two mating types (A1 and A2) were identified among the tested isolates and in all hosts, including in samples collected from the same production area. For example, isolates PCp-142 and PCp-148, both with mating type A1, were found in the same area as PCp-145, with A2. In Nova Friburgo-RJ,

a traditional vegetable-producing region, both mating types were also detected during field surveys carried out in 2008 (**Table 1**). Furthermore, some regions showed only one mating type, such as the isolates PCp-182 and PCp-183, both belonging to the mating type A2, collected in the same area. It was possible to determine the mating type of all tested isolates with 18 of them identified as A1 and 23 of them identified as A2 (**Table 1**).

Determination of the mating types of *Phytophthora capsici* isolates via PCR – No consistent correlation was observed between the phenotypic data of *in vitro* pairings with the results obtained with the primers **Pcap1** and **Pcap2** (**Table 1; Figure 5**). In this sense, isolates PCa-09, PCa-29, PCa-31, and PCp-120 belong to the mating type A1 and isolates PCa-10, PCa-11, PCa-28, PCt-26, PCt-38, PCp -106, PCp-115, PCp-182, PCp-183, PCp-192, to the mating type A2 if we consider the pairing with isolate PCp-192 and PCp-120 as testers A2 and A1 respectively. However, according to the PCR assays the isolates PCp-182 and PC-p183 would be in the mating type A2 (considering the lower band of 500 bp). The isolates PCp-120, PCa-31, PCp-115 amplified the high band of approximately 1000 bp, however, they also amplified an intermediate region of approximately 800 bp, diverging from the expected (A1 with an amplicon of 1000 bp and A2 with 500 bp).

Pathogenicity of *Phytophthora capsici* isolates in bell-pepper fruits – All isolates under evaluation induced disease in bell-pepper fruits, making it possible to visualize mycelium on the infected tissue, four days after inoculation. There were significant differences among the isolates **(Supplementary table 1**). The isolates PCa-31, PCp-183, PCp-167 and PCa-29 showed with higher levels of aggressiveness inducing higher mean lesion diameters in comparison with the other isolates (**Figure 7, panels A and B**).

Pathogenicity of *Phytophthora capsici* isolates in tomato and bell-pepper seedlings – The majority of the isolates induced symptoms in seedlings of bell-pepper ‘Tico’ and tomato

‘Santa Clara’. The symptoms in tomato were stem girdling followed by damping-off. In bell-pepper, crown-rot induced very fast seedling death (**Figure 8**). Only the isolates PCpe-09 and PCt-26 isolates were unable to induce disease in both tomato and bell-pepper seedlings.

Discussion

A precise descrimination of these species based exclusively upon morphological characteristics is difficult because environment conditions can alter the phenotypic characteristics such as sporangium formation and antherid insertion, which may result in misidentification of isolates. Thus, the use of molecular techniques, based on genomic sequences, has been recommended for the adequate characterization of *P. capsici* and other species of the genus.

The close phylogenetic relationship between *P. capsici* and *P. tropicalis* has been verified by different methodological approaches (Donahoo and Lamour, 2008; Aragaki and Uchida, 2001). Homoplasies related to the analysis of ITS-rDNA regions, ribosomal protein 60S L10 (60S), beta-tubulin (ß-tub), elongation factor 1 alpha (EF1α), enolase (Enl), heat shock protein 90 (HSP90), 28S ribosomal DNA (28S), and gene fusion protein (*tigA*) have been already reported in isolates allocated as either *P. capsici* or *P. tropicalis* (Donahoo and Lamour, 2008; Bhai *et al.* 2022). On the other hand, the mitochondrial gene *cox*2 displays a wide array of adequate parameters for a good diagnostic marker for species demarcation, including abundant information available in public databases and high levels of sequence variability (Choudhary *et al.* 2021). Furthermore, the *cox*2 gene sequences of *Phytophtora* isolates are easily and promptly amplified via PCR, producing amplicons with perfect sizes for sequencing analysis.

Here, a precise identification of our collection of isolates was obtained employing the *cox*2 gene information, allowing us to confirm *P. capsici* as the sole causal agent of *Phytophthora*-like disease of a wide array of Solanaceae and Cucurbitaceae crops under Brazilian conditions. This is in agreement with the previous employment of the gene *cox*2 for *Phytophthora* species determination since it can reveal large interspecific and intraspecific divergences (Hudspeth et al. 2000; Martin and Tooley, 2003; Choi et al. 2015), including *P. capsici* and *P. tropicallis*, two closely related species in this genus (Zhang et al. 2004; *Lamour et al.* 2012). Here, the *cox*2 sequence information was suitable for differentiating *P. capsici* from *P. tropicalis* isolates with an adequate bootstrap value, validating and corroborating previous reports in the literature for members of the Clade 2 (Donahoo and Lamour, 2008; Choi *et al.* 2015).

A putative distinct species (named as *P. mexicana*) has been also reported in the Clade 2 (Lamour *et al*. 2012). Even though, *P. mexicana* displays peculiar morphological and physiological characteristics, our *P. capsici* isolates were allocated in the same clade as *P. mexicana* (Brasier *et al.* 2022). In fact, the placement of *P. mexicana* in the same clade as *P. capsici* has already been reported by Oudemans and Coffey (1991) when using isozyme analysis to study the intraspecific and interspecific diversity of the genus *Phytophthora*. In addition, *P. mexicana* and *P. capsici* were not discriminated even using seven informative genomic regions for phylogeny resolution of the genus *Phytophthora* (Blair *et al.* 2008). Therefore, our results are reinforcing previous analyses indicating that both are more likely variants of *P. capsici*.

Interestingly, the topology of *cox*2 gene tree did not differ substantially from previous phylogenetic analyses of the genus *Phytophthora*, with the groupings, in general, agreeing with what was observed employing the entire mitogenome of several members of 19 clades of

the family Peronosporaceae (Winkworth *et al.* 2022). Here, we observed low intraspecific variation in *cox*2 sequence data for the 18 isolates classified as *P. capsici* with the identification of six haplotypes. In addition, the haplotype corresponding to the *cox*2 reference sequence encompassed most of the isolates, suggesting that clonal reproduction drives the population structure of *P. capsici* in Brazil. One atypical/divergent *P. capsici*-like isolate Pcc-004 from cocoa comprised a distinct haplotype.

Sexual reproduction is the main mechanism of variability in *P. capsici* (Lamour *et al.* 2012). In South America, the exclusive presence of A1 was observed in Argentina (Gobena *et al.* 2012), whereas all 27 *P. capsici* isolates collected from different hosts in different regions of the coastal of Peru were identified as mating type A2 (Hurtado-Gonzales et al., 2008). Our *in vitro* analysis of 41 Brazilian isolates indicated 18 isolates from the mating type A1 and 23 from A2. This result is in sharp contrast with previous *in vitro* identification of 130 isolates from several Brazilian states in which 87% of these isolates belonged to the mating type A1 (Paz-Lima, 2006). However, our results may be reflecting the smaller number of collected samples, which were obtained from different geographical locations.

In the present work, isolates of the A1 and A2 mating types were identified occurring in the same producing region. The mating types occurred either singly or concurrently in a subset of the collection sites. The simultaneous occurrence of A1 and A2 isolates has been detected in North American production fields in association with the presence of *P. capsici* oospores (Lamour and Hausbeck, 2000). In Brazil, both mating types were also previously identified, however, not in the same production area (Rêgo and Reifschneider 1982; Marque *et al.* 2004; Paz-Lima, 2006). The simultaneous presence of the two mating types of the pathogen in the same area may allow the occurrence of sexual reproduction, with the formation of oospores and generation of genetic diversity that is associated with greater phenotypic plasticity of

populations of the pathogen, allowing better adaptation to the local environmental conditions and generating variation for relevant traits such as virulence and aggressiveness (Barchenger *et al.* 2018a).

The survival of *P. capsici* in the soil as oospores (Babadoost and Pavon, 2013; Parada-Rojas *et al*. 2021), the low effectiveness of cultural, biological, and chemical control strategies (Saltos *et al.* 2022; Quispe-Quispe et al. 2022; Ma *et al.* 2021) and the lack of commercial cultivars with suitable levels of resistance for many host species (Quesada-Ocampo *et al*. 2016; Petry *et al.* 2017) are among the various challenges for control of this pathogen. In this context, the knowledge about the *P. capsici* diversity across distinct production area is crucial to develop cultivars with broad-spectrum resistance and for the efficient management of the associated diseases (Kousik *et al.* 2022). Furthermore, the presence of distict mating types may hamper the management of diseases caused by *P. capsici* due to long-term survival of the pathogen in the soil conditioned by the occurrence of oospores (Granke *et al.* 2012b).

A total of 11 isolates employed here (PCp-106, PCt-24, PCt-26, PCt-27, PCa-09, PCa-10, PCa-11, PCa-28, PCa-29, PCa-31, PCa-34) were also previously evaluated by Paz-Lima (2006). In that work, the PCt-24, PCa-09, PCa-10 and PCa-11 isolates were classified as mating type A1. In turn, the PCt-24, PCa-10 and PCa-11 isolates were identified as A2 (Paz-Lima, 2006). Of these, only isolate PCa-09 remained as A1. Ribeiro and Bosland (2012) identified the isolates PCp-106, PCp-126, PCp-127, PCp-133, PCt26 as belonging to the mating type A1. However, these isolates were classified here as belonging to A2, suggesting the occurrence of mating type switch in a subset of *P. capsici* isolates stored *in vitro*. Indeed, the phenomenon of mating type switch from A1 to A2 has previously been reported in Taiwan (Barchenger *et al.* 2017; Barchenger *et al.* 2018b). A molecular-genetic evaluation indicated that most of the isolates belonging to mating type A1 were diploid, whereas the A2

isolates were identified as being diploid or triploid, suggesting that the alteration of ploidy could be the genetic mechanism conditioning this characteristic. LOH and DEA has also been linked to the switches from A1 to A2 (Carlson *et al.* 2017; Lamour *et al.* 2012a; Hu *et al.* 2020). In the present work, the PCa-34 isolate showed an atypical switch pattern, switching from A2 to A1, which was previously observed in long-term preserved isolates by Hu *et al.* (2020). However, to the best of our knowledge, this type of switch pattern was not previously detected in Brazilian isolates of *P. capsici*. The present work is, therefore, the first report of this genetic event in Brazilian isolates.

In the original description, the molecular marker of Li *et al*. (2017a) for A1 mating type-specific fragment was determined and analyzed with the BLASTn and BLASTx tools. Here, is the first report of the validation of these molecular markers with a distinct subset of isolates. However, our results were not satisfactory leading us to the conclusion that molecular marker system needs a protocol optimization for the Brazilian isolates. This conclusion was reached based upon inconsistent results displayed between the molecular marker system and our *in vitro* assays. On the other hand, the gene(s) associated with the mating type determination in *P. capsici* are yet unknown, which makes it difficult to develop a set of specific primers for this region. What is known thus far is that a region of 1.6 Mbp is associated with the determination of the mating type, which is called the "mating type region" (MTR) identified by Carlson *et al*. (2017).

The diversity of *P. capsici* isolates can also be assessed via determination of specific patterns of pathogenicity, including host-specificity, organ-specificity, and detection of different avirulent and/or multivirulent pathotypes after inoculation in differential germplasm accessions of certain host plants. In addition, isolates obtained from different hosts can induce different symptoms, which can be influenced by the host, infection site, by environmental

conditions as well as by the stage of development of the plant (Barchenger *et al.* 2018a). In seedlings, damping-off associated with crown rot can kill the plants in two to five days after inoculation, as observed here for the highly susceptible the bell-pepper cultivar 'Tico'. The isolates PCVa-01 and PCpe-09 induced severe symptoms in accessions of *Solanum* (section *Lycopersicon*) in bioassays carry out by Petry *et al*. (2017) whereas, in this study, the isolate PCpe-09 did not cause disease in 'Tico' seedlings or in tomato cultivar 'Santa Clara'. The fact, these distinct patterns of virulence underscore the importance of choosing the appropriate isolate when screening germplasm for resistance.

Granke *et al.* (2012a) observed the presence of different levels of aggressiveness among isolates originated from different hosts. There was no relationship regarding the mating type and the level of aggressiveness in our data, since the more aggressive isolates (PCa-31, PCp183, PCp167 and PCa-29) were identified as A1, A2, A1, and A1, respectively. On the other hand, the subset of less aggressive isolates was composed of both mating types (PCt-26 = A2 and PCt-27 = A1). Previous bioassays indicated that a subset of A2 isolates was less aggressive than A1 isolates (Carlson *et al.* 2017; Paz-Lima, 2006). Interestingly, A1 isolates (diploid) from Taiwan caused more disease than A2 (triploid) isolates (Barchenger *et al*. 2017). According to our results, the isolates originated from bell-peppers caused more severe symptoms than tomato isolates, corroborating the hypothesis that the origin of the isolate in relation to the host can also affect the levels of disease. On the other hand, in this study, most of the isolates, which were not from bell pepper or chili, were maintened for long periods of time (around one decade), which may also have influenced their aggressiveness in immature bell-pepper fruits.

From the point of view of pathogen management and resistance breeding, knowledge of the diversity of *P. capsici* populations is crucial. This is because *P. capsici* isolates can differ in a

wide array of biological traits that may affect their performance as plant pathogens (Ribeiro and Bosland, 2012; Yin *et al.* 2012; Parada-Rojas and Quesada-Ocampo, 2022; Siegenthaler *et al*. 2022). The information about the diversity of this pathogens allows the monitoring of spatial and temporal differences among subpopulations in different agricultural regions (Silvar *et al*. 2006; Sun *et a*l. 2008; Quesada-Ocampo *et al.* 2011; Gobena *et al*. 2012). Therefore, the present characterization of the diversity of *P. capsici* isolates provides important pieces of information towards the sustainable management of this pathogen in Neotropical areas.

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Table 1 – Details of Brazilian *Phytophthora capsici* isolates obtained from Solanaceae and Cucurbitaceae hosts and employed in the present work.

ID	Isolate	Year	Collection site	Original host	Mating type
1	PCa-09	2002	Planaltina–DF	*Cucurbita* sp.	A1
2	PCa-10	2002	Planaltina–DF	*Cucurbita* sp.	**A2**
3	PCa-11	2002	Gama–DF	*Cucurbita maxima*	**A2**
4	PCa-28	2008	Nova Friburgo–RJ	*Cucurbita pepo*	**A2**
5	PCa-29	2008	Nova Friburgo–RJ	*Cucurbita pepo*	A1
6	PCa-31	2006	Uberaba–MG	*Cucurbita moschata*	A1
7	PCa-34	2009	Paracatu–MG	*Cucurbita* sp.	A1
8	PCa-40	2006	Uberaba–MG	*Cucurbita maxima*	A1
9	PCC-02	NA	Uruçuca–BA	*Theobroma cacao*	**A2**
10	PCC-04	1997	Ilhéus–BA	*Theobroma cacao*	A1
11	PCca-02	2019	Camacan–BA	*Theobroma cacao*	A1
12	PCca-01	2019	Camacan–BA	*Theobroma cacao*	A1
13	PCp-106	2006	Barbacena–MG	*Capsicum annuum*	**A2**
14	PCp-109	2006	Morrinhos–GO	*Capsicum annuum*	**A2**
15	PCp-111	2007	Abadiânia–GO	*Capsicum annuum*	A1
16	PCp-115	2007	Abadiânia–GO	*Capsicum baccatum*	**A2**
17	PCp-117	2008	Nova Friburgo–RJ	*Capsicum annuum*	A1
18	PCp-120	2008	Monte Carmelo–MG	*Capsicum annuum*	A1
19	PCp-126	2008	Gama–DF	*Capsicum annuum*	**A2**
20	PCp-127	2008	Rio Manso–MG	*Capsicum annuum*	**A2**
21	PCp-128	2008	Rio Manso–MG	*Capsicum annuum*	A1
22	PCp-133	2008	Nova Friburgo–RJ	*Capsicum annuum*	**A2**
23	PCp-135	2008	Paty do Alferes–RJ	*Capsicum annuum*	A1
24	PCp-138	2008	Nova Friburgo–RJ	*Capsicum annuum*	A1
25	PCp-139	2008	Nova Friburgo–RJ	*Capsicum annuum*	A1
26	PCp-142	2009	Serra Ibiapaba–CE	*Capsicum annuum*	A1
27	PCp-145	2009	Serra Ibiapaba–CE	*Capsicum annuum*	**A2**
28	PCp-148	2009	Serra Ibiapaba–CE	*Capsicum annuum*	A1
29	PCp-153	2009	Ouvidor–GO	*Capsicum annuum*	A1
30	PCp-156	2009	Brazilândia–MG	*Capsicum annuum*	**A2**
31	PCp-159	NA	Bragança Paulista–SP	*Capsicum annuum*	A1
32	PCp-167	NA	Vargem Bonita–DF	*Capsicum annuum*	A1
33	PCp-169	NA	Vargem Bonita–DF	*Capsicum annuum*	**A2**
34	PCp-182	2021	Ceilândia–DF	*Capsicum chinense*	**A2**
35	PCp-183	2021	Ceilândia–DF	*Capsicum chinense*	**A2**
36	PCp-192	NA	Santa Maria–DF	*Capsicum chinense*	**A2**
37	PCp-193	2022	Chã Grande–PE	*Capsicum annuum*	**A2**
38	PCp-196	2022	Camocin de São Felix–PE	*Capsicum annuum*	**A2**
39	PCpe-09	2009	Viçosa–MG	*Cucumis sativus*	**A2**
40	PCt-24	NA	Anapólis–GO	*Solanum lycopersicum*	**A2**

41	PCt-26	2006	Morrinhos–GO	*Solanum lycopersicum*	**A2**
42	PCt-27	2009	Guaraciaba–CE	*Solanum lycopersicum*	A1
43	PCt-38	2010	Brasília–DF	*Solanum lycopersicum*	**A2**
44	PCt-40	NA	Venda Nova do Imigrante–ES	*Solanum lycopersicum*	**A2**
45	PCva-01	2007	Brasília–DF	*Phaseolus vulgaris*	**A2**

Supplementary Table 1. Mean square analysis of inoculation treatment variance for mean diameter of *Phytophthora capsici* mycelium growth in pepper fruits.

	DF	**SQ**	**QM**	**Fc**	**Pr>Fc**
Tratament	9	64.30	7.14	4.69	0.00***
Residual	40	60.90	1.52		
Total	49	125.20			
CV = 20.2%					

"***" Significant at 0,1% probability level, DF = degree of freedom, CV = coefficient of variation.

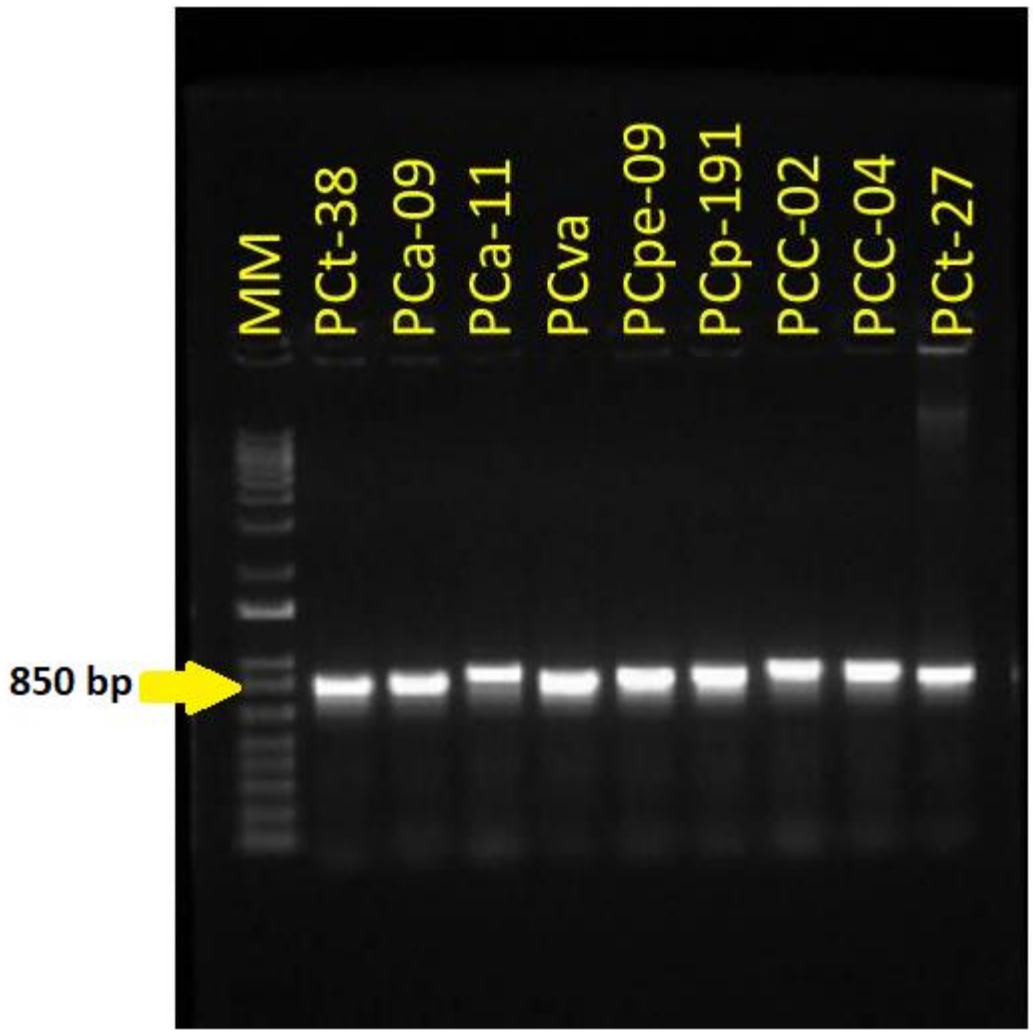

Figure 1 – Agarose gel electrophoresis profile of amplicons (≈ 850 bases pairs) of nine *Phytopthora capsici* isolates (PCt-38, PCa-09, PCa-11, PCva, PCpe-09, PCp-191, PCC-02, PCC-04, and PCt-27) obtained with the PCR primers PF34 (5'–GGC AAA TGG GTT TTC AAG ATC C-3') and PF35 (5'-CCA TGA TTA ATA CCA CAA ATT TCA CTA–3') targeting the *cytochrome-c oxidase subunit* 2 (*cox*2) gene (Hudspeth *et al.* 2000). MM = molecular weight marker 1 Kb Plus (Invitrogen).

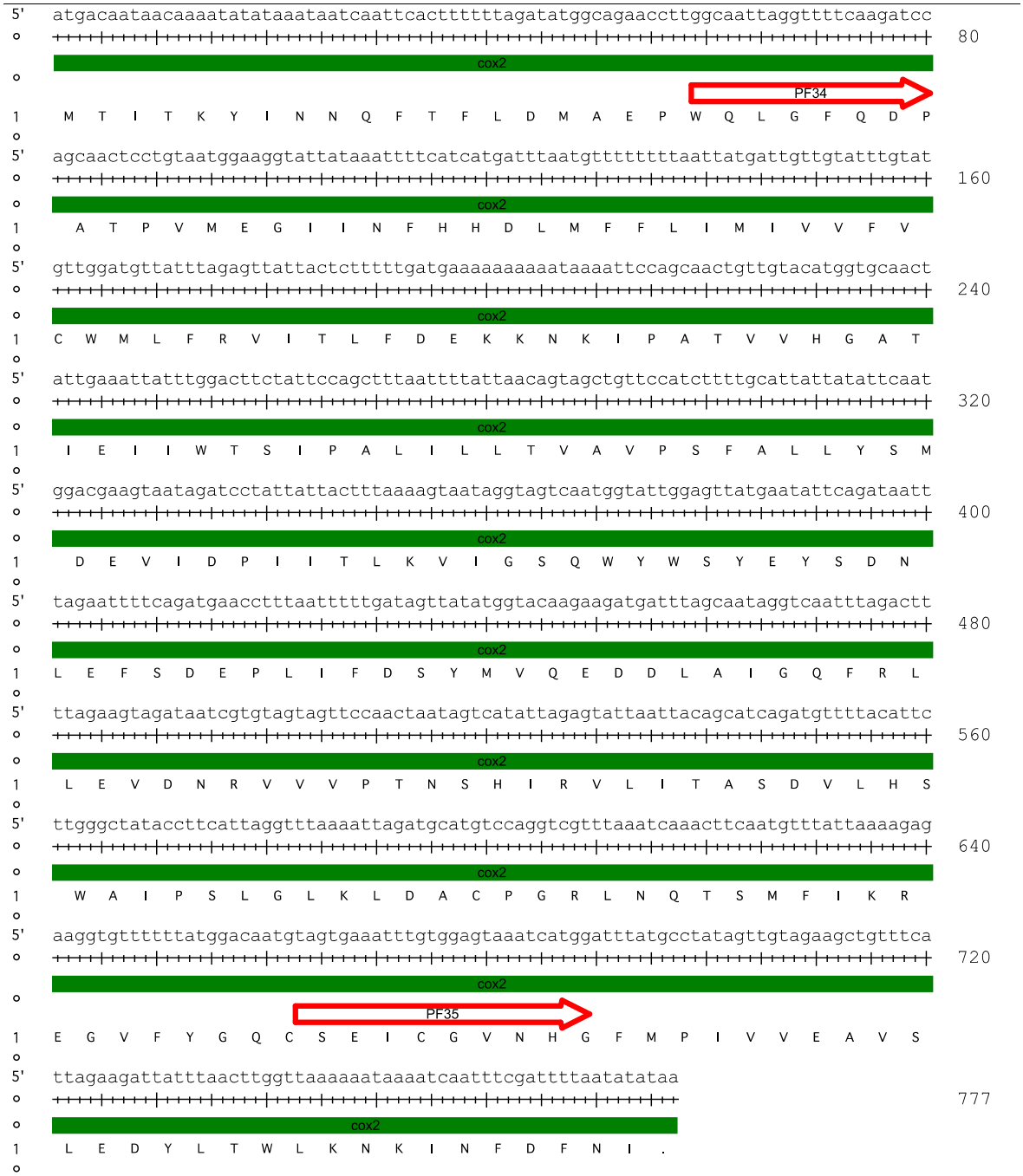

Figure 2 – Sequence of 777 bases of the *cytochrome-c oxidase subunit* 2 (*cox*2) gene from the reference *Phytophthora capsici* (NC_063804) isolate, showing the annelling region of the primers (red arrows) PF34 (5'–GGC AAA TGG GTT TTC AAG ATC C-3') and PF35 (5'-CCA TGA TTA ATA CCA CAA ATT TCA CTA–3') (Hudspeth *et al.* 2000). The PCR generates an amplicon of 634 bases. The bottom line corresponds to the amino acid sequence of the COX 2 protein. The alignment initiated at position 98 (98-100 = codon GGT-Glicyne).

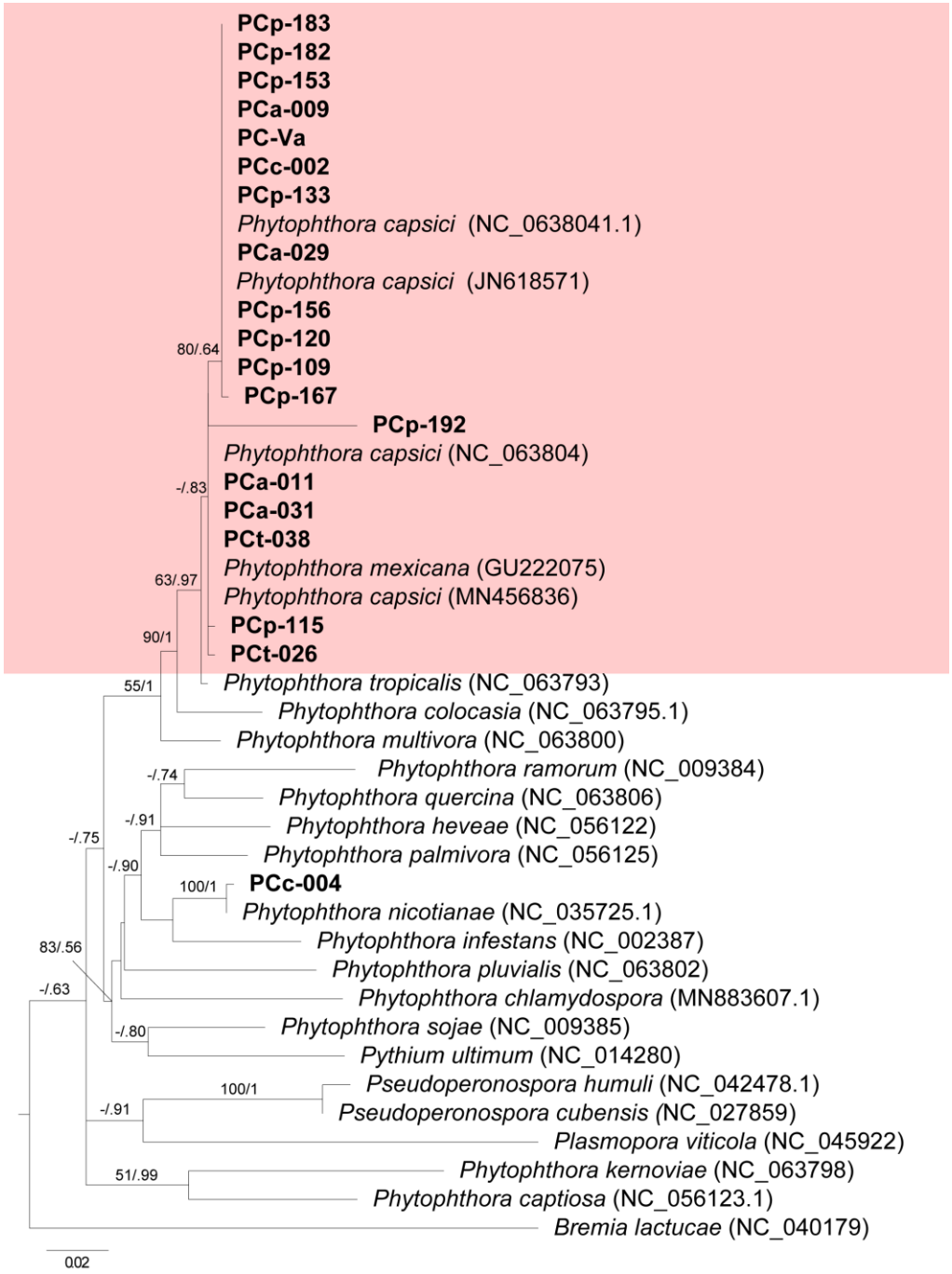

Figure 3 – Maximum likelihood tree inferred from the *cytochrome-c oxidase subunit* 2 (*cox*2) gene alignments of *Phytopthora* species and 18 *P. capsici* isolates employed. Bootstrap support values (≥ 50) and Bayesian posterior Probability values (≥ .50) are indicated at the nodes. The symbol "-" indicates no-significant support or absence of the node. The isolates generated in this study were indicated in **bold**. GenBank accession numbers are shown in parentheses. The Tree is rooted in the midpoint. The scale bar indicates the estimated number of substitutions per site. The isolates are named according to the **Table 1.**

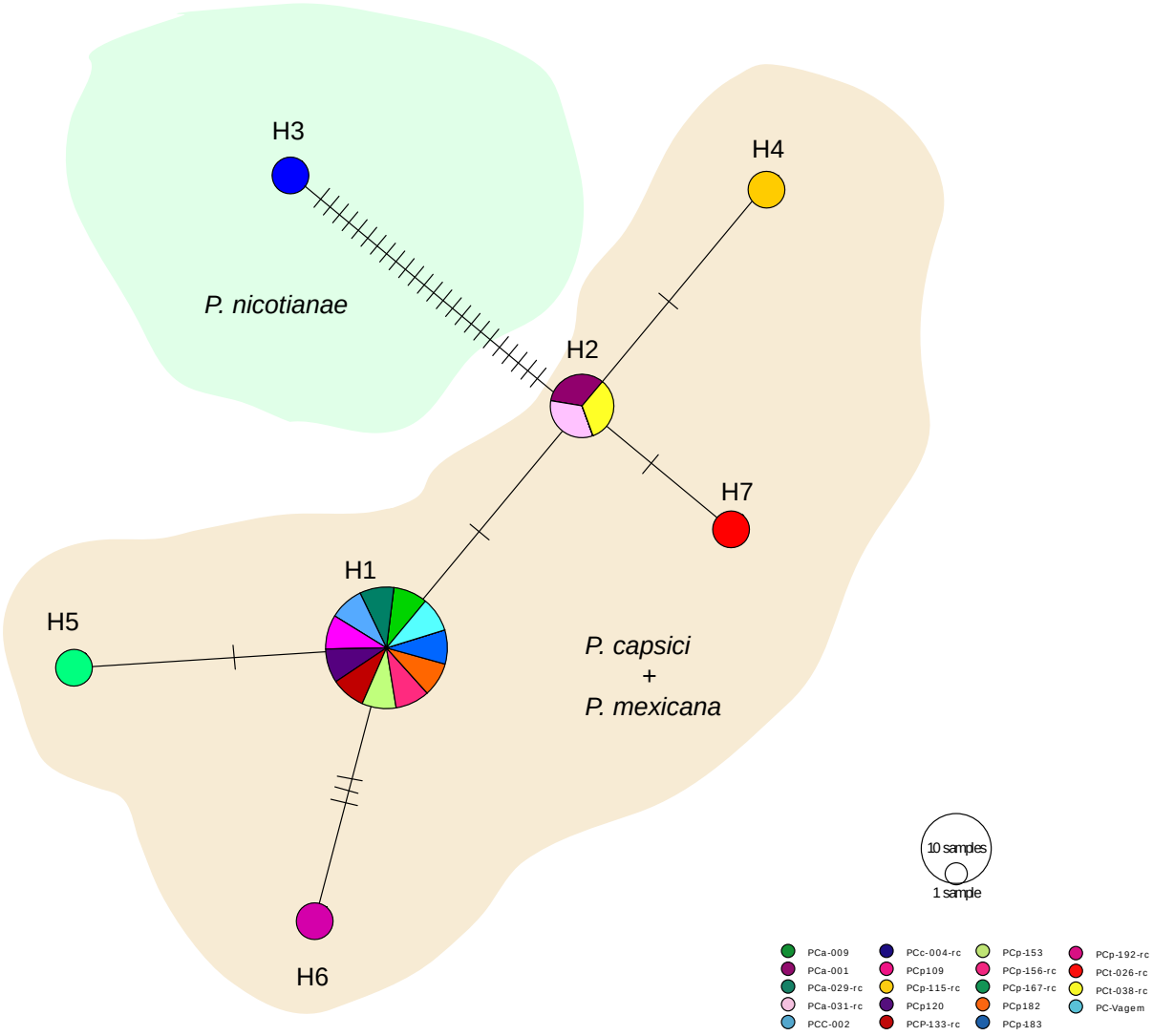

Figure 4 – TCS algorithm-derived haplotype networks of the *cytochrome-c oxidase subunit* 2 (*cox*2) gene from 18 *Phytophthora capsici* isolates and related taxa generated using the PopART program (Leigh and Bryant, 2015) with exclusion of gaps. Each color represents the isolates generated in this study. The sizes of the circles are proportional to the frequency of each haplotype. Hatch markers along the network indicate the corresponding number of mutations. PCa-001 = PCa-011.

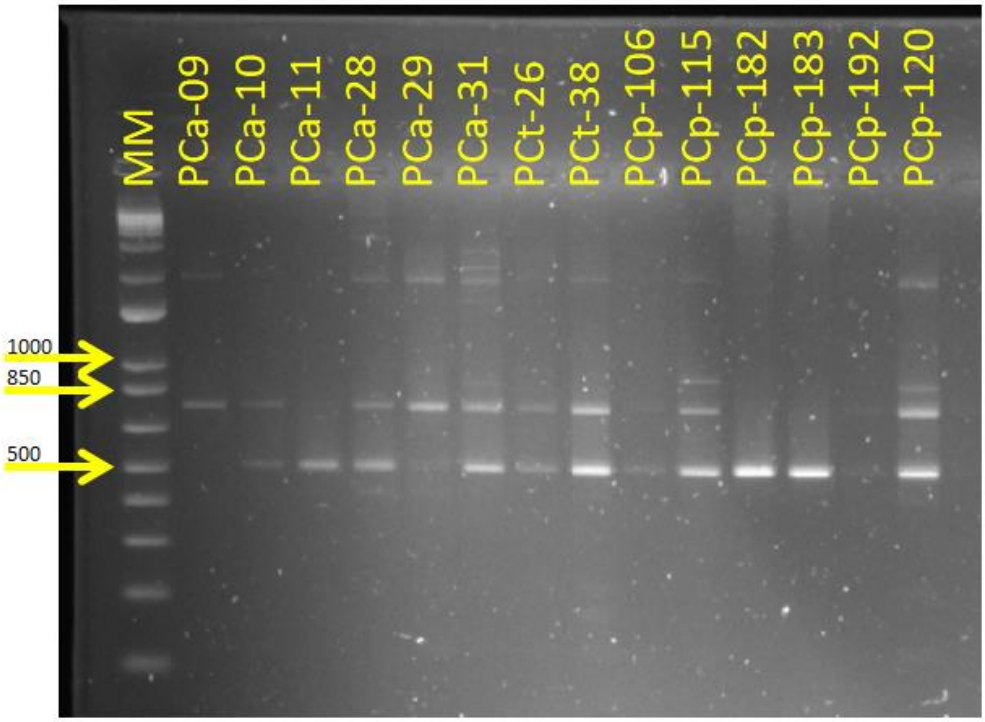

Figure 5 – Validation of a methodology for mating type determination via PCR employing Brazilian isolates of *Phytophthora capsici*. Agarose (1%) gel electrophoresis for amplicon profile of 14 *P. capsici* isolates obtained with the primers **Pcap1** (5'–ACG AGT ACG AGT GCT TGG T–3') and **Pcap2** (5'–TGA GTC TCG AGA CAG AGA G–3') using an annealing temperature of 48°C (Li *et al*. 2017a). MM = molecular weight marker 1 Kb Plus (Invitrogen).

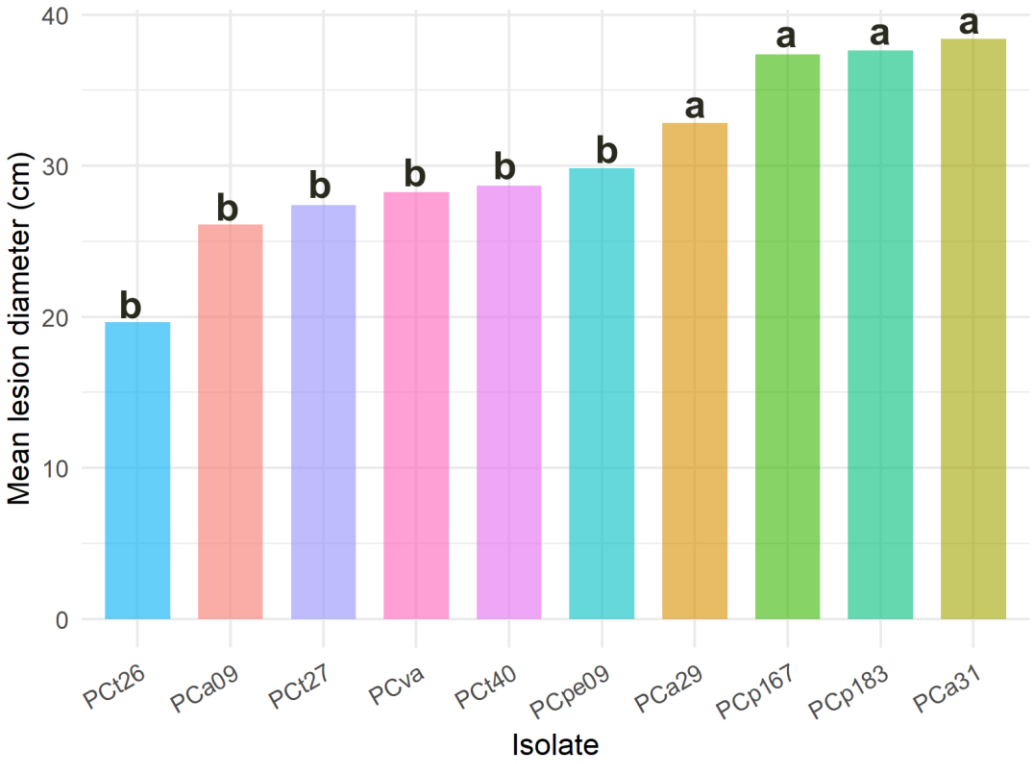

Figure 6 – Differences in mean lesion diameter (cm) induced in *Capsicum annuum* fruits by a subset of *Phytophthora capsici* isolates (**see Table 1**). Means with the same letter are not significantly different according to Scott-Knott test (5%).

Figure 7 – Signs and symptoms observed in bell-pepper (*Capsicum annuum*) fruits after inoculation with *Phytopthora capsici* PCp-167 isolate. **Panel (A):** control assay with water. **Panel (B):** lesions with profuse mycelial growth in the inoculated fruit (five days after inoculation).

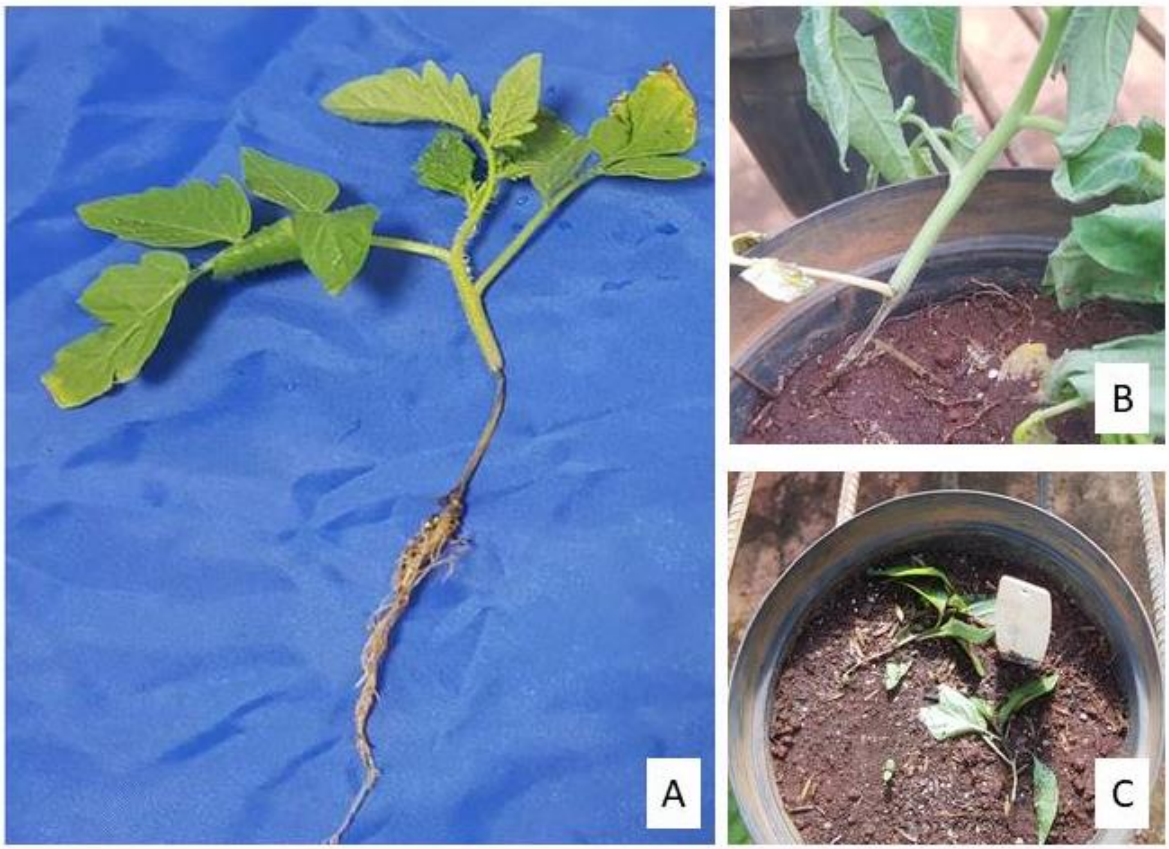

Figure 8 – Symptoms induced by *Phytophthora capsici* in tomato (*Solanum lycopersicum* cv. 'Santa Clara') and bell-pepper (*Capsicum annuum* cultivar 'Tico') seedlings. **Panels A** and **B:** Symptoms of stem girdling and damping-off in 'Santa Clara' seedlings. **Panel C:** Collapse of 'Tico' seedlings.

CAPÍTULO III

Novel sources of broad-spectrum resistance to Neotropical *Phytophthora capsici* isolates in *Solanum lycopersicum* and *S. habrochaites* accessions.

Novel sources of broad-spectrum resistance to Neotropical *Phytophthora capsici* isolates in *Solanum lycopersicum* and *S. habrochaites* accessions.

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Abstract

Phytophthora capsici can induce severe yield losses in multiple crops, including tomatoes (*Solanum lycopersicum*). Sources of broad-spectrum resistance to Neotropical *P. capsici* isolates are not yet available in *Solanum* (*Lycopersicon*) germplasm. We evaluated, in three bioassays, the reaction of 28 accessions against a collection of seven *P. capsici* isolates. *Capsicum annuum* 'Tico' was used as a susceptible control. Inoculations were carried out by depositing a suspension (2×10^4 zoospores/mL) around the crown area of the seedlings and their mortality was evaluated at 14 days after inoculation. Severe symptoms were observed in 'Tico' (100% mortality) in all bioassays. The *Ralstonia*-resistant accession 'Hawaii-7996' displayed superior levels of isolate-specific resistance to four out of six isolates, whereas *S. habrochaites* 'WIR-7924' displayed resistance to five out seven *P. capsici* isolates. Two *S. habrochaites* accessions ('PI-127826' and 'PI-127827') out of 18 displayed an immune-like resistance against two *P. capsici* isolates. The resistant responses of the accessions were not coincident, indicating a potential presence of pathotypes among the isolates. Unstable responses of accessions were observed across the bioassays, indicating either complex inheritance, genetic heterogeneity in accessions of allogamous species or incomplete trait penetrance. Cultivars with broad-spectrum resistance to multiple *P. capsici* isolates is a crucial component for sustainable management of this highly variable pathogen. Hence, the transference of the resistance factors from 'Hawaii-7996' and *S. habrochaites* accessions into elite inbred lines would be a promising breeding strategy towards the development of superior tomato cultivars/rootstocks for Neotropical conditions.

Keywords: Breeding, germplasm, sources of resistance, tomato, oomycete.

Introduction

Phytophthora capsici Leonian (Peronosporales, Pythiaceae) is a biologically and economically important pathogen capable of inducing severe yield losses in a wide array of crops, including tomato – *Solanum lycopersicum* L. (Reis et al., 2018; Saltos et al., 2022). The management of *P. capsici*-induced diseases is difficult due to the ability of the pathogen to survive for long periods of time in the soil (as oospores) and its efficient dispersion by irrigation systems (Café-Filho & Duniway, 1995; Café-Filho et al., 1995; Café-Filho & Duniway, 1996; Moreira-Morrillo et al., 2022). The worldwide detection of populations of *P. capsici* with tolerance to the main active ingredients of pesticides (Café-Filho & Ristaino, 2008; Siegenthaler & Hansen, 2021) and the overall low field effectiveness of chemical control strategies (Saltos et al., 2022) are additional obstacles to the proper management of diseases caused by this oomycete.

Due to the losses induced by *P. capsici*, the deployment of effective technologies for its integrated management is imperative (Lamichhane et al., 2017). The incorporation of genetic resistance into commercial cultivars is one of the most economically viable and ecologically sustainable strategies (Saltos et al., 2021). However, there is a yet small number of studies searching for large-spectrum and phenotypically stable sources of resistance against this highly variable pathogen in many host plants (Reifschneider et al., 1992; Pontes et al., 2014), including tomatoes (Quesada-Ocampo & Hausbeck, 2010; Quesada-Ocampo et al., 2016; Petry et al., 2017). In fact, there are thus far few promising accessions with resistance to *P. capsici* in the genus *Solanum* (section *Lycopersicon*) germplasm (Quesada-Ocampo & Hausbeck, 2010; Quesada-Ocampo et al., 2016; Petry et al., 2017).

The wild tomato *Solanum habrochaites* Knapp & Spooner (former *Lycopersicon hirsutum* Dunal) has emerged as a promising source of resistance to abiotic and biotic constraints, including damages induced by *Phytophthora* species (Abreu et al., 2008; Li et al., 2011; Quesada-Ocampo & Hausbeck, 2010; Nowicki et al., 2012; Quesada-Ocampo et al., 2016; Petry et al., 2017; Seong et al., 2022; Yu et al., 2022). However, the *S. habrochaites* germplasm was not yet properly evaluated against *P. capsici* isolates from Neotropical areas. Inheritance studies with North American isolates indicated that resistance to *P. capsici* in *S. habrochaites* 'LA 407' is more likely controlled by polygenic factors (Quesada-Ocampo et al. 2016), making the incorporation of this trait into elite commercial cultivars more difficult.

Another problem to surpass in the resistance breeding programs is the plasticity of the virulence profile of this pathogen. This feature is characterized by the contrasting isolate-specific reactions as well-documented in many host accessions of the *P. capsici–Capsicum* pathosystem (Silvar et al., 2006; Granke et al., 2012, Ribeiro & Bosland, 2012).

From the tomato breeding standpoint, a major initiative for the host genetic management of this highly variable pathogen is to intensify the evaluation of a greater number of accessions within the germplasm of the genus *Solanum* (section *Lycopersicon*) with special emphasis on the promising wild species *S. habrochaites*. In the present work, we carried out three bioassays under greenhouse conditions with the objective of evaluating the reaction of 28 accessions of *Solanum* (section *Lycopersicon*) against seven Neotropical isolates of *P. capsici*, in search of new and useful sources of resistance for tomato breeding programs.

Material and Methods

***Solanum* (*Lycopersicon*) accessions and *P. capsici* isolates employed in the bioassays –** Three biossays were carried out at the greenhouses of the National Center for Vegetable Crops Research (CNPH), Embrapa Vegetable Crops (Embrapa Hortaliças), Brasília–DF, Brazil, aiming to evaluate the reaction of a diverse germplasm of 28 *Solanum* (section *Lycopersicon*) accessions (Table 1) against a collection of seven *P. capsici* isolates obtained from different hosts and from distinct geographic regions (Table 2). The identity of the isolates was confirmed via morphological analysis and sequencing of the *cox*2 gene (data not shown). This subgroup of isolates was selected based upon their ability to induce severe symptoms in a subset of cultivated tomato accessions evaluated in previous screening trials (Petry et al., 2017). In all three bioassays, the pathogenicity of the isolates and the viability of the inoculum were confirmed by simultaneously inoculating three pots (with three seedlings each) of the susceptible control *Capsicum annuum* L. cultivar ‘Tico’.

Inoculum preparation – Pure cultures of each isolate were transferred to Petri dishes containing 20% V8 agar medium (100 mL V8 juice, 1.5 g calcium carbonate, 10 g of agar, and 400 mL distilled water). Isolates were kept in growth chambers for three days in the dark, and then for five days under continuous light (25 ± 2 °C) to induce profuse sporangia production. Subsequently, sterile distilled water (20 mL) was added to each Petri dish, and

these were transferred to 6 °C for one hour, followed by 30 minutes at 25 °C aiming to induce the production of zoospores. The zoospores suspension was homogenized and filtered through sterile gauze. Zoospore concentration was adjusted to 2 *x* 10^4 zoospores mL^{-1} with the aid of the Neubauer's chamber.

Seedling production and inoculation – Seeds of all *Solanum* (*Lycopersicon*) accessions were sown in commercial styrofoam trays with 128 cells, filled with sterile Plantmax® commercial substrate (Costa et al., 2013). When the seedlings were displaying two pairs of fully expanded true-leaves (25 days after sowing), they were removed from the tray cells and transplanted onto 1.5 L plastic pots (three plants per pot) containing the same substrate. Seedlings were then inoculated with each isolate (separately), via deposition around the crown area of an aliquot of 3 mL of the suspension.

Bioassay #1: Assessment against three *P. capsici* isolates of *Solanum* (*Lycopersicon*) accessions identified as promising sources of resistance in previous assays – Evaluation of a subset of 11 *Solanum* (*Lycopersicon*) accessions (Figure 1, Figure 2, and Supplementary Table 4) previously identified as the most promising sources of resistance to *P. capsici* (Petry et al. 2017) was challenged with three novel *P. capsici* isolates ('PCp-182', 'PCp-191', and 'PCp-192'). The experiment was carried out in a completely randomized design in a 12 *x* 3 factorial scheme (11 accessions plus the susceptible control challenged with three isolates). Three replicates (three pots with three plants each) were used for each accession. The pathogenicity of the isolates and the viability of the inoculum were confirmed by the simultaneous inoculation of three pots with seedlings of *C. annuum* 'Tico' (three seedlings in each). For an improved evaluation of the interactions among pathogen isolates, host accessions and environmental conditions, the area under the disease progress curve (AUDPC) was calculated in the bioassay #1. Seven days after inoculation, evaluations were initiated considering the incidence of collapsed/dead plants. Four evaluations at seven-day intervals were carried out and the average values of the AUDPC were calculated according to the formula of Campbell & Madden (1990). AUDPC mean values were submitted to analysis of variance (ANOVA) and compared using the Scott-Knott test ($P \leq 0.05$). All statistical analyses of the bioassay #1 were performed using the epifitter and Scott-Knott packages in R statistical software https://www.R-project.org/.

Biossay #2: Evaluation of a core collection of 18 *S. habrochaites* accessions against two *P. capsici* isolates – A core collection of 18 *S. habrochaites* accessions (Table 3 and Supplementary Table 5) was evaluated against two *P. capsici* isolates ('PCp-183' and 'PCp-192'). In the bioassay #2, we included two selections genetically related to *S. habrochaites* 'LA 407' (the original germplam source 'PI-251304' and a selection of this accession from Ecuador). *Solanum habrochaites* 'LA 407' was previously identified as the most promising source of resistance against North American isolates of *P. capsici* (Quesada-Ocampo & Hausbeck, 2010; Quesada-Ocampo et al. 2016). The experiment was carried out in an 18 *x* 2 factorial scheme (17 accessions plus a susceptible control challenged with two pathogen isolates). Three replicates (three pots with three plants each) were used for each accession.

Biossay #3: Assessment of three of the most promising *Solanum* (*Lycopersicon*) accessions identified in the bioassays #1 and #2 against five *P. capsici* isolates – The bioassays #1 and #2 served as the primary screening of the most promising accessions. Hence, the bioassay #3 was a corroboration of the stability of the results observed in the two previous bioassays as well as in previous work (Petry et al., 2017). We arbitrarily selected three of the most promising resistant accessions plus the accession 'CNPH 1143' that was used as a tomato control (Table 4 and Supplementary Table 6) These accessions were then challenged with five isolates viz. 'PCa-028', 'PCa-031', 'PCp-182', 'PCp191', and 'PCt-038' (Table 2). The experiment was carried out in a 5 *x* 5 factorial scheme (three *Solanum* accessions plus the susceptible tomato control and the susceptible sweet-pepper control challenged with five isolates). Three replicates (three pots with three plants each) were used for each accession.

Statistical analyses of seedling mortality in all bioassays – The evaluation criterion was disease incidence, which was expressed as the percentage of *Solanum* (*Lycopersicon*) seedling mortality (= number of collapsed or dead plants over the total) at 14 days after inoculation (= the best evaluation time as indicated by the AUDPC data from the bioassay #1). A generalized linear model (GLM) was proposed to describe the effect of *P. capsici* isolates on the *Solanum* (*Lycopersicon*) accessions since mortality of the seedlings may or may not occur (i.e. a binary variable with a binomial distribution). Disease incidence data was then expressed by the estimated seedling mortality (SM%) = (MT-MC/100-MC)*100. Where: MT = % of seedling mortality in the accessions (treatments) and MC = % of seedling mortality in the susceptible control. Then, the SM data were grouped by the Scott-Knott test ($P \leq 0.05$). Accessions with SM above 50% were classified as susceptible and below this value as

resistant. The data were analyzed using the stats package of the R statistical software (R Core Team 2022; https://www.R-project.org/).

Results

Bioassay #1: Assessment against three *P. capsici* isolates of *Solanum* (*Lycopersicon*) accessions identified as promising sources of resistance in previous assays – Significant differences were observed across accessions, isolates and accession-isolate interactions (Supplementary Table 1). All three *P. capsici* isolates ('PCp-182', 'PCp-191', and 'PCp-192') induced 100% mortality in seedlings of *C. annuum* 'Tico' (employed as a susceptible control), indicating that they fully preserved their pathogenicity. Moreover, all isolates induced damping-off symptoms in individual seedlings of at least nine out of 11 *Solanum* (*Lycopersicon*) accessions (Figure 1). A subgroup of four accessions displayed isolate-specific, immune-like responses against the isolates 'PCp182' and 'PCp192', but no accession displayed similar reaction to the isolate 'PCp191' (Figure 1 and Supplementary Table 4). The inbred line 'Hawaii-7996' displayed an immune-like response (0% mortality) against two (PCp-182', and 'PCp-192') out of three isolates, whereas *S. habrochaites* 'WIR-7924' showed similar immune-like reaction to the isolate 'PCp-192'. *Solanum lycopersicum* 'Micro-Tom' was asymptomatic to the isolate 'PCp-182'. Significant differences were detected among the 11 *Solanum* (*Lycopersicon*) accessions in the overall analysis of their reaction against all three isolates, based in GLM.

Based upon AUDPC values, the 11 *Solanum* (*Lycopersicon*) accessions were classified into three reaction groups (Figure 2). The accessions 'Guardião', 'Ohio 4013', 'Ponderosa Red', 'IPA-2', and 'CNPH 1143' were not different from *C. annuum* 'Tico' (susceptible control), being classified as susceptible. 'Guardião' displayed inferior response in this combined analysis due to its extreme susceptibility (100% mortality) to one ('PCp191') out of the three isolates employed in the bioassay #1. In relation the the isolates, 'PCp-191' induced the highest mortality rates, and it was significant different from the other two isolates for this biological trait (Supplementary Table 2). On the other hand, the accessions 'WIR-7924', 'CNPH 1048', 'Micro-Tom' and 'Hawaii-7996' displayed more stable levels of partial resistance across isolates and they were classified into the resistant group (Figure 2).

Biossay #2: Evaluation of a core collection of 18 *Solanum habrochaites* accessions against two *Phytophthora capsici* isolates – Significant differences were observed among isolates,

accessions, and isolate-accession interactions in the bioassay #2 (Supplementary Table 3). All *P. capsici* isolates induced 100% seedling mortality in *C. annuum* 'Tico'. The accessions *S. habrochaites* 'WIR-3611', 'PI-126445' and 'CNPH 1034' when inoculated with the isolate 'PCp-192' and 'CNPH 1112' and 'PI-128650' when inoculated with the isolate 'PCp-183', displayed overall low levels of partial resistance performing similarly to the susceptible check *C. annuum* 'Tico'. Four *S. habrochaites* accessions ('PI-365934', 'WIR-7924', 'PI 127826', and 'PI 127827') showed significant higher levels of resistance against the two isolates in a combined analysis (Table 3). The accessions 'PI 127826' and 'PI 127827' were the only two displaying an immune-like resistance against both *P. capsici* isolates (Figure 3 and Table 3). The seedling mortality was higher after inoculation with the isolate 'PCp-183' (Table 3). 'PCp-183' induced 10% seedling mortality in 'WIR-7924', which displayed an immune-like response to the isolae 'PCp-192'. Significant isolate-accession interactions were also observed in 'PI-126445', 'PI-134418', and 'PI-126449' with 100, 70, and 80% seedling mortality, respectively, when inoculated with the isolate 'PCp-192'. On the other hand, 'PI-126445', 'PI-134418', and 'PI-126449' displayed 100, 20, and 20% seedling mortality, respectively, when inoculated with the 'PCp-183' isolate (Figure 3 and Supplementary Table 5).

Biossay #3: Assessment of three of the most promising *Solanum* (*Lycopersicon*) accessions identified in the bioassays #1 and #2 against five *P. capsici* isolates – A subset of two *S. lycopersicum* accessions ('Micro-Tom' and 'Hawaii-7996') and one *S. habrochaites* accession ('WIR-7924') identified as the most promising sources of isolate-specific resistance in bioassays #1 and #2 were again evaluated in the bioassay #3 against five isolates ('PCp-182', 'PCp-191', 'PCa28', 'PCa31', and 'PCt38') obtained from different hosts (Table 2). *Solanum lycopersicum* 'CNPH 1143' was used as a susceptible control due to its inferior performance in bioassay #1. Significant differences were observed among accessions, isolates and accession-isolate interactions (Supplementary Table 3). The accessions 'Hawaii-7996', 'Micro-Tom' and *S. habrochaites* 'WIR-7924' showed the best results when tested with this collection of five isolates (Table 4 and Figure 4). However, no immune-like response was observed after inoculation with the 'PCp-182' isolate that was able to induce seedling mortality varying from 0.44 to 0.89 (Figure 4; Supplementary Table 6). The isolates 'PCt-38', 'PCa31', and 'PCa28' induced the lowest seedling mortalities in this subgroup of accessions, while the isolate 'PCp-182' induced the highest seedling mortality, mainly in the accessions

‘CNPH 1143’, ‘Micro-Tom’, and ‘WIR-7924’, showing the broadest virulence profile among the five isolates evaluated in bioassay #3 (Table 4 and Figure 4).

Discussion

In the germplasm encompassing accessions of the cultivated tomato (*S. lycopersicum*), the inbred line ‘Hawaii-7996’ displayed superior levels of isolate-specific resistance to four out of five isolates. ‘Hawaii-7996’ is genetically related to ‘CNPH 1048’. These two inbred lines are distinct single-plant selections from ‘Hawaii-7996’, the original source of bacterial wilt (*Ralstonia* species) resistance (Wang et al., 1998; Lopes et al., 2015; Albuquerque et al., 2021). Therefore, it was expected that ‘Hawaii-7996’ and ‘CNPH 1048’ would display similar responses to *P. capsici* isolates due to their close genetic relationship. In fact, similar patterns of reaction of both accessions were observed in the bioassay #1. Hence, the accession ‘Hawaii-7996’ can be considered a useful source of multiple resistant factors to distinct soil pathogens. Interestingly, *S. lycopersicum* ‘Micro-Tom’, which is a model tomato cultivar for genetic and genomic studies (Kobayashi et al., 2014), displayed high level of isolate-specific (‘PCc182’) resistance in the bioassay #1 in combination with variable levels of partial or rate-reducing resistance (*sensu* Nelson, 1978) to other isolates. However, the resistance derived from ‘Micro-Tom’ was found to be unstable since heterogenous responses were observed across the bioassays #1 and #3.

The genetic diversity of the cultivated tomato is quite narrow due to the process of domestication as well as the effects of selection carried out in commercial breeding programs. In this regard, the search for additional sources of resistance in germplasm collections of wild *Solanum* (*Lycopersicon*) species is a valuable breeding strategy (Khazaei & Madduri, 2022). *Solanum habrochaites* is an outstanding source of resistance to a wide array of pathogens (Foolad et al., 2008; González-Arcos et al. 2018; Jedelská et al., 2021; Lian et al., 2022; Miao et al. 2022), including resistance against *Phytophthora* species (Abreu et al., 2008; Li et al., 2011; Nowicki et al., 2012; Quesada-Ocampo & Hausbeck, 2010; Quesada-Ocampo et al., 2016; Petry et al., 2017). However, the present study was the first comprehensive evaluation of *S. habrochaites* germplasm against Neotropical *P. capsici* isolates carried out thus far. *Solanum habrochaites* ‘WIR-7924’ displayed superior levels of resistance to five out seven *P. capsici* isolates, whereas the *S. habrochaites* ‘PI-127826’ and ‘PI-127827’ were the only two among a core germplasm collection of 18 accessions of this wild species that displayed an

immune-like resistance against two *P. capsici* isolates in the bioassay #2. This immune-like reaction to a subset of *P. capsici* isolates, suggests the potential presence of qualitative (monogenic or oligogenic) resistance, which is in contrast with previous studies using distinct *S. habrochaites* resistance sources (Quesada-Ocampo et al., 2016). Unstable reactions of a subgroup of *S. habrochaites* to distinct isolates were observed and they can be partly explained by some level of residual heterozygosity, which is a genetic characteristic of allogamous accessions of this wild species (Markova et el., 2016). It is also important to highlight that this novel subset of *S. habrochaites* accessions displayed higher levels of resistance than two selections of *S. habrochaites* 'LA 407' (= 'CNPH 1286' and 'CNPH 1772'), which was an accession previously detected as the best source of resistance to *P. capsici* isolates from North America (Quesada-Ocampo & Hausbeck, 2010; Quesada-Ocampo et al., 2016). Here, we were also able to detect highly susceptible *S. habrochaites* accessions that might be employed as susceptible parental lines in interspecific inheritance studies.

The genetic/phenotypic variability of both *Solanum* (*Lycopersicon*) accessions and *P. capsici* isolates in conjuction with environmental conditions are major factors influencing host-pathogen interactions in this pathosystem. Understanding the genetic diversity of *P. capsici* populations is fundamental for the breeding programs aiming for the development of phenotypically stable resistant cultivars (Barchenger et al. 2018). *Phytophthora capsici* is able to evolve rapidly, complicating the identification of reliable sources of resistance (Fan et al., 2022). For this reason, to screen breeding lines and germplasm accessions with a large collection of *P. capsici* isolates is a crucial procedure due to the potential presence of pathotypes/physiological races of the pathogen, as observed in in pathosystem involving *Capsicum* species (Ribeiro & Bosland, 2012; Lee et al., 2021).

Here, we observed significant differences for accession-isolate interactions in all three bioassays employing a set of seven *P. capsici* isolates. The reactions of the accessions to this collection of isolates were not coincident, suggesting the presence of potential physiological races or pathotypes. Illustrative examples of these highly contrasting isolate-specific reactions in our bioassays are the interactions of 'WIR-7924' with the isolate 'PCp-182' in the bioassays #1 and #3 and 'WIR-7924' with the isolate 'PCa28' in the bioassay #3; 'Guardião' with the isolate "PCp 191' in the bioassay #1; 'Hawaii-7996' interaction with the isolate 'PCp-192' in the bioassay #3; 'PI-126445' with the isolate 'PCp192' in the bioassay #2 and

‘PI-134418’ that was resistant to ‘PCp-192’ but highly susceptible to ‘PCp-183’ also in bioassay #2, reinforcing the hypothesis of the presence of distinct races/pathotypes.

Little is yet known about the environmental parameters that might affect the interaction of *P. capsici* with many of its hosts (Nawaz et al., 2018). Inoculum concentration and host plant age may play important roles in the expression of the level of resistance as observed in the *Capsicum–P. capsici* pathosystem (Reifschneider et al., 1986). In this sense, the inoculum concentration used under our conditions (2×10^4 zoospores per mL) and the employment of young seedlings are in accordance with the methodology that was efficiently employed in previous assays (Petry et al., 2017). In addition, our screening conditions proved to be efficient as indicated by of the highly susceptible reaction of *C. annuum* ‘Tico’ seedlings. This susceptible control displayed 100% mortality after inoculation with all isolates across all the three bioassays, indicating a minimal impact of the potential variation in the environmental conditions.

In this context, the detected instabilities of the reactions of the *Solanum* (*Lycopersicon*) accessions across our bioassays might be mainly due to differences in the virulence profiles of the *P. capsici* isolates. In addition, it is also important to emphasize that some unstable host responses might be result of combination of factors including, the presence of genetic heterogeneity in *S. habrochaites* accessions as well as the potential oligogenic or polygenic control of the resistance (which expression is more affected by environmental factors). This type of “partial resistance” or “rate reducing resistance” (*sensu* Nelson, 1978) may explain the variation observed in the seedling mortality in a subgroup of accessions across the three bioassasys. Another possibility is the incomplete penetrance of the resistance factors, resulting in phenotypic instability of the associated trait, which is a common feature of many disease resistance genes in tomato (Boiteux & Giordano, 1993). A third and less likely hypothesis is the genetic changes of the isolates after their successive *in vitro* subculturing, which may lead to modifications in their ability to induce disease. Therefore, experiments to screen host germplasm for *P. capsici* resistance must comprise, whenever possivel, a large set of isolates.

In conclusion, we detected novel and potentially useful sources of resistance to an array of *P. capsici* isolates in *Solanum* (*Lycopersicon*) germplasm. The incorporation into commercial cultivars of broad-spectrum resistance factors is a key breeding component to establish

sustainable management strategies of this highly variable pathogen. In addition, this large-scale germplasm screening work will allow the potential identification of alternative broad-spectrum resistance to *P. capsici* with less complex genetic control (i.e. monogenic or oligogenic). Hence, the transfer of the resistance factors from 'Hawaii-7996' and *S. habrochaites* accessions into elite inbred lines would be a promising breeding strategy towards the development of superior tomato cultivars/hybrids/rootstocks for Neotropical conditions.

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Declarations

Conflicts of interest: The authors declare that there is no conflict of interest.

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Table 1 – Twenty-eight (28) *Solanum* (section *Lycopersicon*) accessions of the germplasm collection of the National Center for Vegetable Crops Research (CNPH), Embrapa Vegetable Crops (Embrapa Hortaliças) which was evaluated for their reaction to *Phytophthora capsici* isolates in three controlled greenhouse bioassays. *Capsicum annuum* 'Tico' (susceptible control) was included in all assays.

Code	***Solanum* (section *Lycopersicon*) species and cultivars**
'Hawaii-7996'	*Solanum lycopersicum* 'Hawaii-7996' – J.W. Scott (University of Florida)
CNPH 1048	*Solanum lycopersicum* (a Brazilian selection of 'Hawaii-7996')
CNPH 0040	*Solanum lycopersicum* 'IPA-2'
CNPH 0315	*Solanum lycopersicum* 'Atkinson'
CNPH 0342	*Solanum lycopersicum* 'Ponderosa Red'
CNPH 1143	*Solanum lycopersicum* 'Brazilian landrace of unknown pedigree'
CNPH 1284	*Solanum lycopersicum* 'Micro-Tom'
CNPH 0266	*Solanum lycopersicum* 'Manitoba'
CNPH 0698	*Solanum lycopersicum* 'Ohio 4013'
'Guardião'	*Solanum lycopersicum* (Commercial rootstock hybrid)
CNPH 0416	*Solanum habrochaites* PI-126445 (Selection from Brazil)
CNPH 0417	*Solanum habrochaites* PI-126449 (Selection from Brazil)
CNPH 0420	*Solanum habrochaites* PI-127826
CNPH 0421	*Solanum habrochaites* PI-127827
CNPH 0423	*Solanum habrochaites* PI-134417
CNPH 0424	*Solanum habrochaites* PI-134418
CNPH 0605	*Solanum habrochaites* 'WIR-3611'
CNPH 0929	*Solanum habrochaites* 'WIR-7924'
CNPH 1034	*Solanum habrochaites* 'Hortus Botanicus of the Neederlands' (unknown pedigree)
CNPH 1112	*Solanum habrochaites* 'Landrace of unknown pedigree'
CNPH 1122	*Solanum habrochaites* 'L 03684' (AVRDC) – *Phytophthora infestans* resistant line
CNPH 1286	*Solanum habrochaites* PI-251304 (= LA 407; selection from Ecuador)
CNPH 1287	*Solanum habrochaites* PI-126445 (Selection from Peru)
CNPH 1288	*Solanum habrochaites* PI-247087 (Ecuador)
CNPH 1289	*Solanum habrochaites* PI-128650 (Chile)
CNPH 1290	*Solanum habrochaites* PI-126449 (Selection from Chile)
CNPH 1772	*Solanum habrochaites* PI-251304 (= LA 407; original source)
CNPH 1773	*Solanum habrochaites* PI-365934 (= LA 1353)

Table 2 – List of *Phytopthora capsici* isolates obtained from different hosts and regions of Brazil and employed in the one or more bioassays reported in the present study.

Isolate code	**Collection site**	**Original host species**
'PCp-182'	Ceilândia–DF	*Capsicum chinense*
'PCp-183'	Ceilândia–DF	*Capsicum chinense*
'PCp-191'	Brasília–DF	*Capsicum chinense*
'PCp-192'	Santa Maria–DF	*Capsicum chinense*
'PCa-028'	Nova Friburgo–RJ	*Cucurbita pepo*
'PCa-031'	Uberaba–MG	*Cucurbita moschata*
'PCt-038'	Brasília–DF	*Solanum lycopersicum*

Table 3 – Estimated seedling mortality for accessions of 18 *Solanum habrochaites* and *Capsicum annuum* 'Tico' (susceptible control) inoculated with two *P. capsici* isolates and induced seedling mortality by *P. capsici* isolates ('PCp-183'and 'PCp-192') in the **bioassay #2**.

Accessions	SM%*		Reaction class	Isolate	Mortality	
'Tico'	1.00	a	Susceptible	PCp183	0.61	A
'CNPH 1034'	0.96	a	Susceptible	PCp192	0.54	B
'PI-128650'	0.91	a	Susceptible			
'WIR-3611'	0.91	a	Susceptible			
'CNPH 1112'	0.87	a	Susceptible			
'PI-247087'	0.79	a	Susceptible			
'PI-134417'	0.75	a	Susceptible			
'L 03684'	0.66	b	Susceptible			
'PI-126449' (Brazil selection)	0.62	b	Susceptible			
'PI-126445' (Brazil selection)	0.62	b	Susceptible			
'LA 407' (Ecuador selection)	0.58	b	Susceptible			
'PI-126445' (Peru selection)	0.58	b	Susceptible			
'PI-126449' (Chile selection)	0.54	b	Susceptible			
'PI-134418'	0.42	c	Resistant			
'LA 407' (original source)	0.42	c	Resistant			
'LA 1353' = 'PI-365934'	0.16	d	Resistant			
'WIR-7924'	0.04	d	Resistant			
'PI-127827'	0.00	d	Resistant			
'PI-127826'	0.00	d	Resistant			

*Seedling mortality (**SM**%) data were grouped by the Scott-Knott test ($P \leq 0.05$). Accessions with SM above 50% were classified as susceptible (S) and below this value as resistant (R).

Table 4 – Estimated seedling mortality of *Solanum* (section *Lycopersicon*) accessions and *Capsicum annuum* 'Tico' (susceptible control) inoculated with five *P. capsici* isolates and estimated mortality rates of *P. capsici* isolates ('PCp182', 'PCp191', 'PCa28', 'PCa31', and 'PCt38') in the **bioassay #3**.

Accessions	SM%*		Reaction class	Isolates	Mortality	
CNPH 1143	0.52	a	Susceptible	PCp182	0.70	A
'Micro-Tom'	0.36	b	Resistant	PCp191	0.48	B
'WIR-7924'	0.34	b	Resistant	PCa28	0.30	C
'Hawaii-7996'	0.22	b	Resistant	PCa31	0.18	C
				PCt38	0.15	C

*Seedling mortality (**SM**%) data were grouped by the Scott-Knott test ($P \leq 0.05$). Accessions with SM above 50% were classified as susceptible (S) and below this value as resistant (R).

Supplementary Table 1 – Deviance analysis for seedling mortality of ten *Solanum* (section *Lycopersicon*) accessions inoculated with three *Phytophthora capsici* isolates in the **bioassay #1**.

	Degree of Freedom (DF)	**Deviance**	**Residual DF**	**Residual Deviation**	**Pr(>Chi)**
Null			323.00	448.85	
Accession	11	91.05	312.00	357.80	0.00***
Isolate	2	41.45	310.00	316.36	0.00***
Accession–isolate	22	73.78	288.00	242.57	0.00***

*** Significantly different at the 0.01 level (tabulated the chi-square).

Supplementary Table 2 – Deviance analysis for seedling mortality of 18 *Solanum habrochaites* accessions and *Capsicum annuum* 'Tico' (susceptible control) inoculated with two *Phytophthora capsici* isolates in the **bioassay #2**.

	Degree of Freedom (DF)	**Deviance**	**Residual DF**	**Residual Deviation**	**Pr(>Chi)**	
Null			455.00	622.56		
Accession	18	229.56	437.00	393.00	0.00	***
Isolate	1	5.51	436.00	387.49	0.02	*
Accession–isolate	18	52.81	418.00	334.69	0.00	***

***Significantly different at the 0.01 (chi-square tabulated); *Significantly different at the 0.05 (chi-square tabulated).

Supplementary Table 3 – Deviance analysis for seedling mortality of four *Solanum* (section *Lycopersicon*) accessions inoculated with five *Phytophthora capsici* isolates in the **bioassay #3.**

	Degree of Freedom (DF)	Deviance	Residual DF	Residual Deviation	Pr(>Chi)	
Null			179	236.58		
Accession	3	9.77	176	226.81	0.021	*
Isolate	4	32.29	172	194.52	0.000	***
Accession–isolate	12	35.98	160	158.55	0.000	***

***Significantly different at the 0.001 (chi-square tabulated); *Significantly different at the 0.05 (chi-square tabulated).

Supplementary Table 4 – Estimated seedling mortality rates of *Solanum* (section *Lycopersicon*) accessions and *Capsicum annuum* 'Tico' (susceptible control) inoculated with three *Phytophthora capsici* isolates ('PCp-182', 'PCp-191', and 'PCp-192') in the **bioassay #1**.

Accessions	PCp182		PCp191		PCp192	
	SM%*	**RC***	**SM%***	**RC**	**SM%***	**RC**
'IPA-2'	0.11	R	0.89	S	0.56	S
'Manitoba'	0.44	R	0.67	S	0.67	S
'Atkinson'	0.22	R	1.00	S	0.33	R
'Ponderosa Red'	0.22	R	1.00	S	0.33	R
'Ohio 4013'	0.44	R	0.56	S	0.89	S
'WIR-7924'	0.67	S	0.11	R	0.00	R
'CNPH 1048'	0.00	R	0.44	R	0.44	R
'CNPH 1143'	0.78	S	1.00	S	0.56	S
'Micro-Tom'	0.00	R	0.33	R	0.22	R
'Guardião'	0.11	R	1.00	S	0.11	R
'Hawaii-7996'	0.00	R	0.33	R	0.00	R
'Tico'	1.00	S	1.00	S	1.00	S

*Seedling mortality (**SM**%) data were grouped by the Scott-Knott test ($P \leq 0.05$). Accessions with SM above 50% were classified as susceptible (S) and below this value as resistant (R).

Supplementary Table 5 – Estimated seedling mortality rates for *Solanum habrochaites* accessions and *Capsicum annuum* 'Tico' (susceptible control) inoculated with two *Phytophthora capsici* isolates ('PCp183' and 'PCp192') in the **bioassay #2**.

Accessions	PCp183		PCp192	
	SM%*	RC	SM%*	RC
'PI-126445' (Brazil selection)	0.25	R	1.00	S
'PI-126449' (Brazil selection)	0.83	S	0.42	R
'PI-127826'	0.00	R	0.00	R
'PI-127827'	0.00	R	0.00	R
'PI-134417'	0.92	S	0.58	S
'PI-134418'	0.67	S	0.17	R
'WIR-3611'	0.83	S	1.00	S
'WIR-7924'	0.08	R	0.00	R
'CNPH 1034'	0.92	S	1.00	S
'CNPH 1112'	1.00	S	0.75	S
'L 03684'	0.75	S	0.58	S
'LA 407' (Ecuador selection)	0.58	S	0.58	S
'PI-126445' (Peru selection)	0.50	R	0.67	S
'PI-247087'	0.83	S	0.75	S
'PI-128650'	1.00	S	0.83	S
'PI-126449' (Chile selection)	0.83	S	0.25	R
'LA 407' (original source)	0.42	R	0.42	R
'LA 1353'	0.25	R	0.08	R
'Tico'	1.00	S	1.00	S

*Seedling mortality (**SM**%) data were grouped by the Scott-Knott test ($P \leq 0.05$). Accessions with SM above 50% were classified as susceptible (S) and below this value as resistant (R).

Supplementary Table 6 – Estimated seedling mortality of *Solanum* (section *Lycopersicon*) accessions inoculated with five *Phytophthora capsici* isolates (PCa28, PCa31, PCp182, PCp191, and PCt38) in **the bioassay #3**.

Accessions	**PCa28**		**PCa31**		**PCp182**		**PCp191**		**PCt38**	
	SM%*	**RC***	**SM%***	**RC**	**SM%***	**RC**	**SM%***	**RC**	**SM%***	**RC**
'Micro-Tom'	0.00	R	0.33	R	0.78	S	0.44	R	0.33	R
'CNPH 1143'	0.44	R	0.33	R	0.89	S	0.78	S	0.22	R
'WIR-7924'	0.78	S	0.00	R	0.67	S	0.11	R	0.11	R
Hawaii-7996	0.00	R	0.11	R	0.44	R	0.56	S	0.00	R

*Seedling mortality (**SM**%) data were grouped by the Scott-Knott test ($P \leq 0.05$). Accessions with SM above 50% were classified as susceptible (S) and below this value as resistant (R).

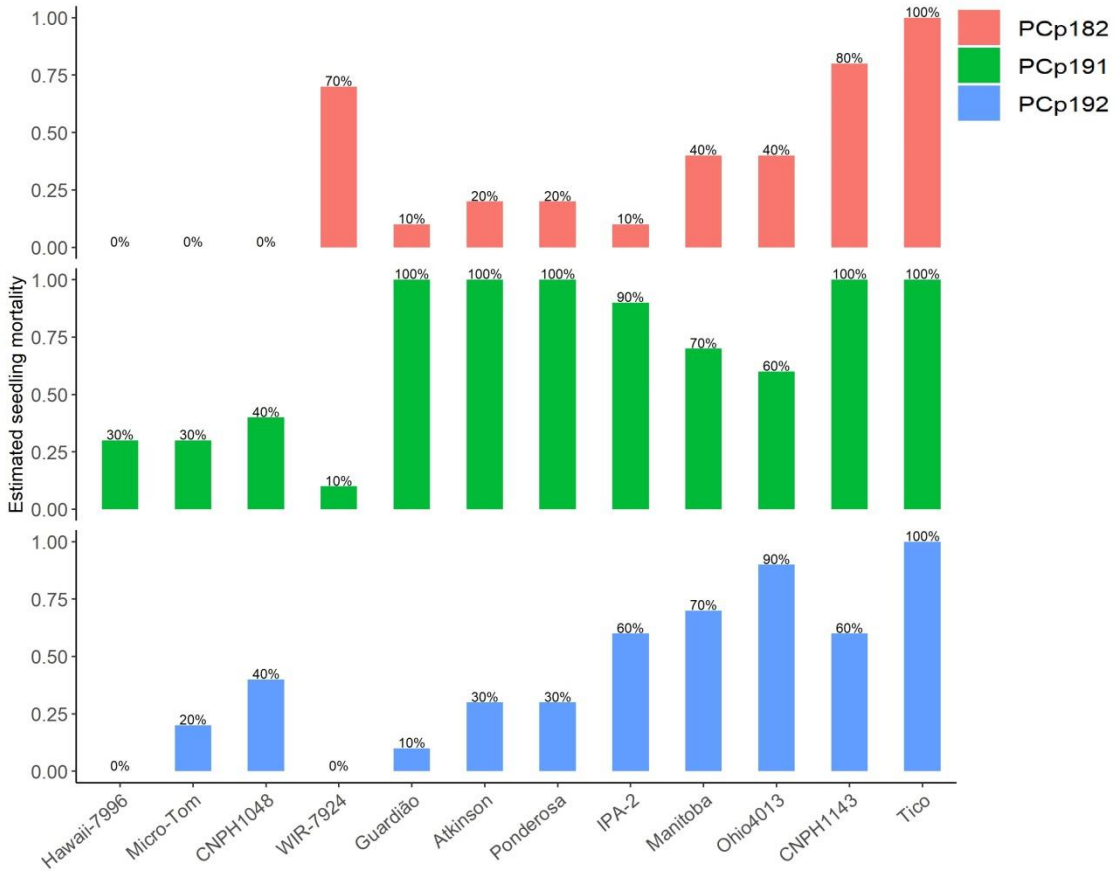

Figure 1 – Estimated seedling mortality of 11 *Solanum* (section *Lycopersicon*) accessions (see Table 1) and *Capsicum annuum* 'Tico' (susceptible control) inoculated with three *Phytophthora capsici* isolates 'PCp-182' (red panel), 'PCp-191' (green panel), and 'PCp-192' (blue panel) in the **bioassay #1**.

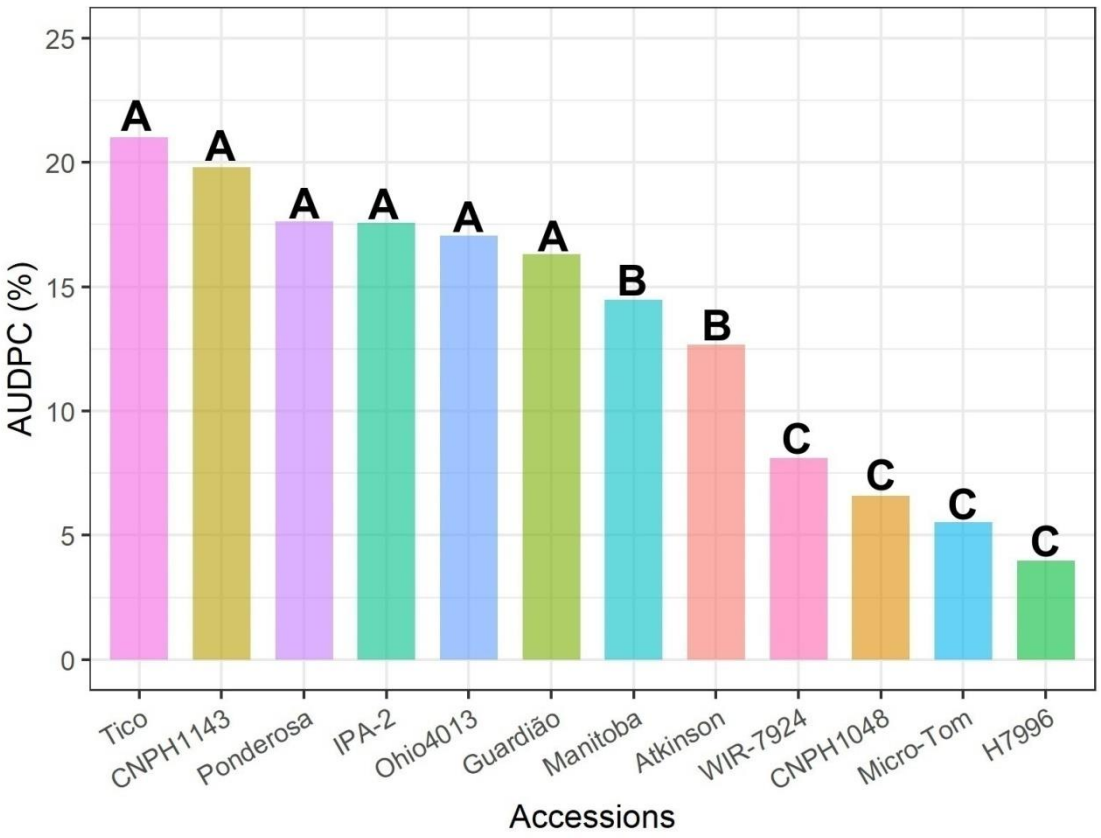

Figure 2 – Average test (Scott-Knott) estimation of the Area Under Disease Progress Curve (AUDPC) of 11 *Solanum* (section *Lycopersicon*) accessions (see Table 1) and *Capsicum annuum* 'Tico' (susceptible control) after inoculated with three *Phytophthora capsici* isolates ('PCp-182', 'PCp-191', and 'PCp-192') in the **bioassay #1**. Means followed by the same letter do not differ by Scott-Knott test ($P \leq 0.05$).

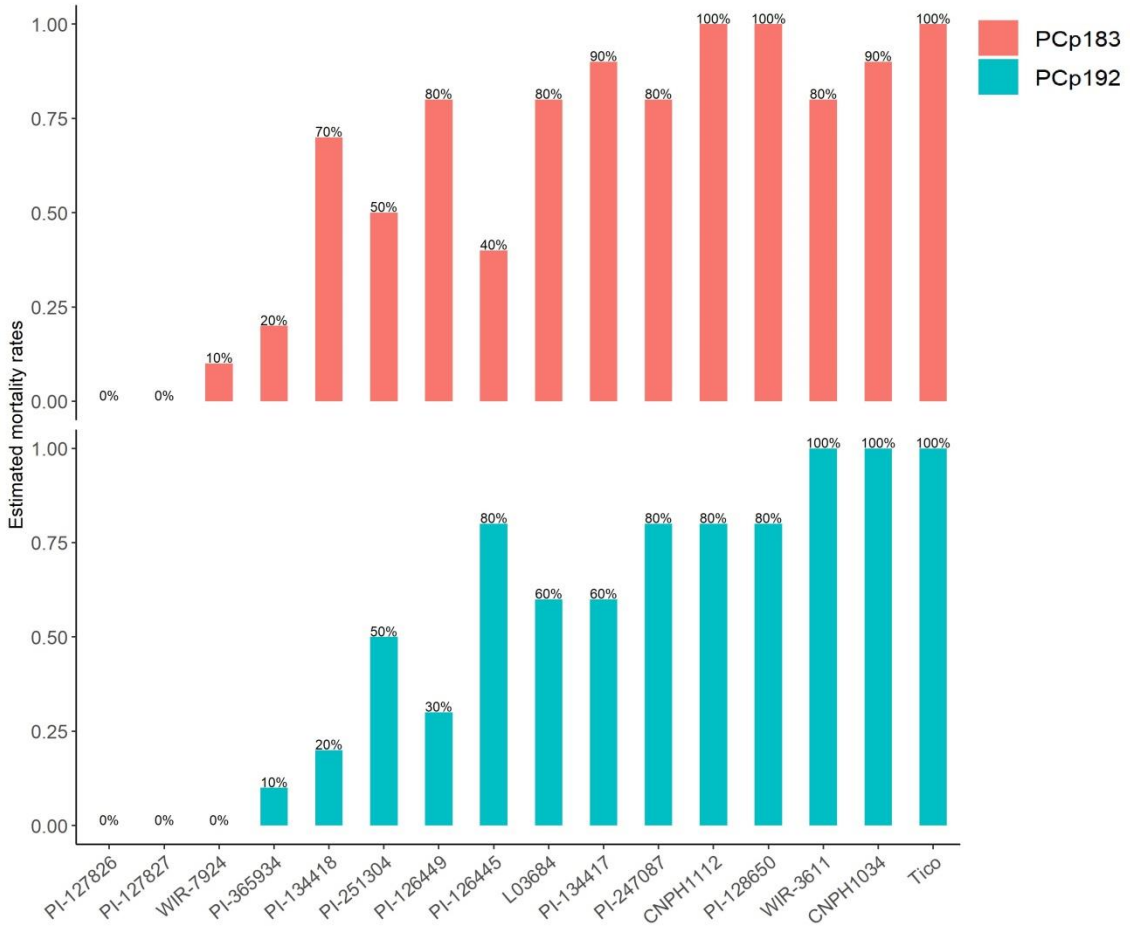

Figure 3 – Estimated seedling mortality of 18 *Solanum habrochaites* accessions (see Table 1) and *Capsicum annuum* 'Tico' (susceptible control) in response to the inoculation of two *Phytophthora capsici* isolates 'PCp-183' (red panel) and 'PCp-192' (blue panel) in the **bioassay #2**.

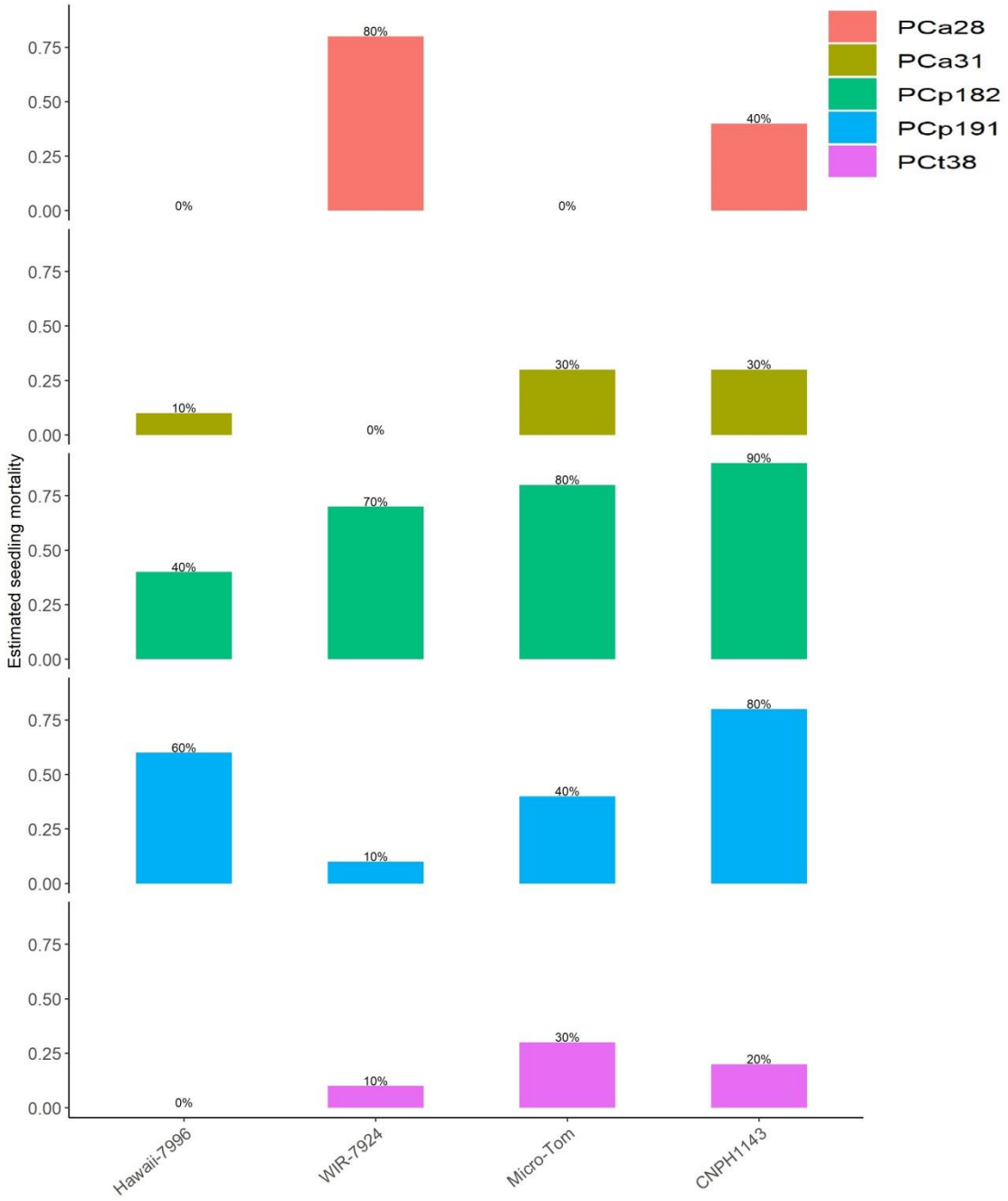

Figure 4 – Estimated seedling mortality of *Solanum habrochaites* 'WIR-7924'(= purple panel), *Solanum lycopersicum* 'Micro-Tom' (blue panel); *S. lycopersicum* 'Hawaii-7996' (green panel) and *S. lycopersicum* (red panel) in response to the inoculation with five *Phytophthora capsici* isolates ('PCa-028', 'PCa-031', 'PCp-182', 'PCp191', and 'PCt-038') in the **bioassay # 3**.

CAPÍTULO IV

Assessment of a suitable panel of differential host accessions for pathotype identification in the *Phytophthora capsici – Solanum* (*Lycopersicon*) pathosystem

Assessment of a suitable panel of differential host accessions for pathotype identification in the *Phytophthora capsici – Solanum* (*Lycopersicon*) pathosystem.

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Abstract

Phytophthora capsici is a major soil-borne pathogen of tomatoes (*Solanum lycopersicum*). The management of *P. capsici* is difficult mainly due to the lack of resistant cultivars and the broad virulence profile of this pathogen. Thus far, no extensive work has investigated the patterns of interaction of *Solanum* (*Lycopersicon*) accessions and Neotropical *P. capsici* isolates. Pathogen isolates (from different hosts and geographic regions of Brazil) were used to evaluate the reaction of *Solanum* (*Lycopersicon*) accessions previously identified as resistant to one or more isolates. The cultivar 'Santa Clara' displayed a "universal" susceptible reaction (100% mortality), whereas *S. lycopersicum* 'Hawaii-7996' and *S. habrochaites* 'PI-127826' and 'PI-127827' displayed resistance against a wide array of isolates. Eight pathotypes were identified in a sample of ten isolates according to their unique interaction patterns with nine host accessions, which are suggested as differential *Solanum* (*Lycopersicon*) genotypes in pathotype determination bioassays. Hence, pathotypes can be identified via simple bioassays based upon the capacity of a given isolate to "break-down" a specific set of genes of this unique germplasm, providing a more precise panorama of the field-occurring pathogen variability. Inheritance and mapping studies of these pathotype-specific resistance factors in each accession will facilitate their incorporation into commercial cultivars.

Keywords: tomato, *Solanum lycopersicum*, germplasm, sources of resistance, breeding.

Introduction

Tomato (*Solanum lycopersicum* L. = *Lycopersicon esculentum* Mill.) is one of the most important vegetable crops around the world (Bauchet and Causse, 2012). *Phytophthora capsici* Leonian can impose severe negative effects especially for processing tomatoes, but also for fresh-market tomatoes in the early stages of crop development (Lamour *et al.* 2012; Saltos *et al.* 2022). The cultural management of this oomycete is difficult due to an array of biological and genetic characteristics of this pathogen (Nawaz *et al.* 2018; Barwell *et al.* 2021; Saltos *et al.* 2022), including its broad host range (Lamour *et al.* 2012; Pontes *et al.* 2014, Barboza *et al.* 2017; Reis *et al.* 2018); production of oospores (which allows pathogen overwintering in the soil in the absence of host plants) and its efficient field dispersion, notwithstanding the irrigation system (Café-Filho and Duniway, 1995; Café-Filho *et al.* 1995; Café-Filho and Duniway, 1996; Moreira-Morrillo *et al*. 2022).

In this scenario, the employment of host cultivars with genetic resistance to this pathogen would be the most sustainable component in integrated management systems (Lamichhane *et al.* 2017; Saltos *et al.* 2021). However, the development of cultivars with broad-spectrum resistance is challenging due to the genetic and phenotypic plasticity of *P. capsici* (Lamour *et al*. 2012). This oomycete has both asexual and sexual reproduction systems, contributing to the continuous emergence of subpopulations with remarkable variability (Café-Filho and Ristaino, 2008; Castro-Rocha *et al.* 2016; Reyes-Tena *et al.* 2019b; Reyes-Tena *et al.* 2021; Fan *et al.* 2022). Striking differences in the virulence profiles among isolates have been reported in the pathosystem *P. capsici* – *Capsicum* (Lee *et al.* 2010; Granke *et al*. 2012; Ribeiro and Bosland, 2012; Reyes-Tena *et al.* 2019a; Lee *et al.* 2021). Altogether, 45 potential races (= pathotypes) have been reported in *Capsicum* in North America (Ribeiro and Bosland, 2012; Jiang *et al*., 2015; Barchenger *et al*., 2018b); 11 in Korea (Lee *et al.* 2010) and ten in Mexico (Reyes-Tena *et al.* 2019a) and two in Spain (Silvar *et al*. 2006). The presence of numerous races/pathotypes in *Capsicum*-infecting isolates have been attributed to distinct genetic mechanisms, including sexual reproduction, loss of heterozygosity as well as point mutations and genetic recombination (Lamour e*t al.* 2012; Ribeiro and Bosland, 2012; Jiang *et al.* 2015; Barchenger *et al.* 2018a).

Differences of the virulence profiles among *P. capsici* isolates have been also observed in their interactions with *Solanum* (*Lycopersicon*) accessions (Quesada-Ocampo *et al*. 2016;

Petry *et al.* 2017a). More recently, Gonçalves-Pereira *et al*. (2023) also detected significant accession-isolate interactions in bioassays employing seven Brazilian *P. capsici* isolates and 28 *Solanum* (*Lycopersicon*) accessions. In the pathogen side, significant differences were observed across a collection of isolates in relation to their levels of host-inducing mortality among *Solanum* (*Lycopersicon*) accessions, reinforcing the hypothesis of the presence of distinct races/pathotypes (Gonçalves-Pereira *et al.* 2023). This hypothesis was also reinforced by the identification of a subgroup of *S. lycopersicum* and *S. habrochaites* accessions displaying both broad-spectrum and isolate-specific resistance/susceptibility. Furthermore, the isolate-specific reactions were not always coincident, suggesting once more the presence of distinct pathotypes among the evaluated sample of *P. capsici* isolates. Gonçalves-Pereira *et al.* (2023) proposed that a subset of informative *Solanum* (*Lycopersicon*) accessions displaying sharp contrasting responses to *P. capsici* should be evaluated as potential pathotype/race differentials employing a larger collection of isolates.

From the breeding standpoint, a more precise knowledge about the natural variation in the virulence phenotypes of *P. capsici* is vital for successful development of cultivars with phenotypically stable (= large-spectrum) resistance to this pathogen (Ribeiro and Bosland, 2012; Reyes-Tena *et al.* 2019a). In a situation involving a highly dynamic pathogen (Lamour *et al.* 2012), one of the major breeding targets would be the incorporation into elite cultivars of multiple pathotype-specific resistance genes in conjunction with broad-spectrum resistance genes (Jiang *et al.* 2015). Nevertheless, no extensive work has yet investigated or cataloged the variability of the virulence profiles of Neotropical *P. capsici* isolates in tomatoes as well as the patterns of interaction with specific sources of resistance detected in *Solanum* (*Lycopersicon*) germplasm.

In the present work, a collection of *P. capsici* isolates (from different hosts and geographic regions) was used to assess a subgroup of *Solanum* (*Lycopersicon*) accessions previously detected with higher levels of resistance to one or more Brazilian and North American isolates of this oomycete (Quesada-Ocampo *et al.* 2016; Petry *et al.* 2017a; Gonçalves-Pereira *et al.* 2023). Another major objective of the present work was to identify the potential presence of well-defined pathotypes in *P. capsici* – *Solanum* (*Lycopersicon*) pathosystem under Brazilian conditions.

Material and methods

Plant material – Three bioassays were conducted with the aim of evaluating the reaction of a collection of *S. lycopersicum* and *S. habrochaites* accessions (**Table 1**) to an array of virulent *P. capsici* isolates (**Table 2 and Table 3**). These accessions were previously detected by their contrasting levels of resistance/susceptibility to one or more isolates of this oomycete (Quesada-Ocampo *et al.* 2016; Petry *et al.* 2017a; Gonçalves-Pereira *et al.* 2023). The bioassays were conducted at the greenhouse facilities of the National Center for Vegetable Crops Research (CNPH), Embrapa Vegetable Crops (Embrapa Hortaliças) in Brasília–DF, Brazil. Seedlings of the tomato cultivar 'Santa Clara' and *Capsicum annuum* cultivar 'Tico' were included in all assays to verify inoculum viability. The seeds of the accessions were sown in 128-cell Styrofoam trays, filled with sterile Plantmax® substrate. When the seedlings were displaying two pairs of fully expanded true-leaves (≈25 days after sowing), they were removed from the tray cells and transplanted onto 1.5 L plastic pots (three plants per pot) containing sterile Plantmax® substrate for subsequent *P. capsici* inoculation bioassays.

Inoculum preparation and seedling inoculation – Pure cultures from each of the 30 *P. capsici* isolates (**Table 2**) were transferred to Petri dishes containing 20% V8 agar medium (100 mL V8 juice, 1.5 g calcium carbonate, 10 g of agar, and 400 mL distilled water). Isolates were kept in growth chambers for three days in the dark, and then for five days under continuous light (25 ± 2 °C) to induce sporangia production. Afterwards, sterile distilled water (20 mL) was added to each Petri dish, and these were transferred to 6 °C for one hour, followed by 30 minutes at 25 °C with the objective of inducing a substantial production of zoospores. The zoospore suspension was homogenized and filtered through sterile gauze. Zoospore concentration was adjusted (2×10^4 zoospores mL^{-1}) with the aid of a Neubauer's chamber. The *Solanum* (*Lycopersicon*) seedlings were allowed to recover from the transplant stress (≈ 5 days) and then inoculated with a suspension of each isolate (separately) via deposition in the crown area of an aliquot of 3 mL. The viability of the isolates was confirmed by simultaneous inoculation of 'Santa Clara' and *C. annuum* 'Tico' (susceptible control) seedlings.

Bioassay #1 – A subset of six *Solanum* (*Lycopersicon*) accessions ('Santa Clara', 'Ponderosa', 'WIR 7924', 'Micro-Tom', 'CNPH 1143', and 'Hawaii-7996') was challenged with 20 *P. capsici* isolates. The experiment was carried out in a completely randomized

design in a 6 *x* 14 factorial scheme where six accessions were challenged with 14 virulent isolates (**Fig. 1 and Fig. 2**). Three replicates (three pots with three plants each) were used for each accession.

Bioassay #2 – A subset of four *Solanum* (*Lycopersicon*) accessions (*S. habrochaites* 'PI 127826' and 'PI 127827' and the tomato cultivars 'Ponderosa' and 'Santa Clara') was challenged with 13 *P. capsici* isolates (**Fig. 3 and Fig. 4**). The experiment was carried out in a completely randomized design in a 4 *x* 13 factorial scheme (five accessions challenged with 16 isolates). Three replicates (three pots with three plants each) were used for each accession.

Bioassay #3 – Four *S. habrochaites* accessions ('PI 127826', 'PI 127827', 'LA407', and 'WIR 7924') and five *S. lycopersicum* accessions ('CNPH 1143', 'Micro-Tom', 'H-7996', 'Santa Clara', and 'Ponderosa') accessions were challenged with ten virulent *P. capsici* isolates. The experiment was carried out in a completely randomized design in a 9 *x* 10 factorial scheme (nine accessions challenged with ten isolates). Three replicates (three pots with three plants each) were used for each accession.

Accession-isolate interactions and statistical analyses – At 14 days after inoculation, the disease incidence/mortality (= frequency of either collapsed or dead plants) was evaluated by counting the number of symptomatic seedlings over the total number of plants assessed in each of the three replications. This evaluation time was found to be the most appropriated evaluation time to discriminate distinct resistance levels among accessions (Gonçalves-Pereira *et al*., 2023). The incidence/mortality was chosen as a single parameter for evaluation since the main disease symptom was collar-rot with subsequent plant death, making the use of a rating scale unrealistic. All data obtained from the counting of diseased plants in relation to healthy plants were analyzed by a generalized linear model (GLM) to investigate the effect of the main factors (accessions, isolates, and their interactions) in the three bioassays. The mortality rates for the accession-isolate interactions were calculated using the following formula: Estimated **seedling mortality** (**SM** %) = (MT-MC/100-MC) * 100. Where: MT = % mortality in of the accessions under evaluation and MC = % mortality in the susceptible control. The SM values were submitted to analysis of variance and their means were compared by the Scott-Knott test ($P \leq 0.05$). Pathotypes were identified by quantitative differences in the percentage of dead plants for each accession-isolate interaction across all three bioassays. Differences in the estimated mortality rates were employed to determine the

virulence profile of each isolate. All analyses were carried out using the stats package of the R statistical software (R Core Team 2022; https://www.R-project.org/).

Results

Seventeen (17) out of 30 *P. capsici* isolates (**Table 2**) were able to induce 100% mortality in *C. annuum* 'Tico' (susceptible control). A subet of these virulent isolates was evaluated in the three bioassays.

In **bioassay #1**, a subgroup of 14 isolates caused disease in the six *Solanum* (*Lycopersicon*) accessions ('Santa Clara', 'Ponderosa', 'WIR 7924', 'Micro-Tom', 'CNPH 1143', and 'Hawaii-7996'). The deviance analysis detected statistically significant effects among accessions, among isolates and for accession-isolate interactions (**Supplementary Table 1**). Considering the combined analysis of **SM** as a function of isolates, 'PCp-182' displayed the best performance (overall seedling mortality of 80%). The isolates with the lowest performance ('PCt38' and 'PCp187') induced **SM** values ranging from 28 to 31% (**Figure 1**). Considering the combined analysis of the **SM** values according to the accessions, significant differences were observed among accessions, with the establishment of three groups with 'Hawaii-7996' displaying the best performance among the host accessions (**Supplementary Table 2**). The individual seedling mortality dataset (considering accession-isolate interactions) is shown in **Figure 2**. The best performing isolates ('PCp-182' and 'PCp-153') induced **SM** values ranging from 57 to 80%. In this sense, if we consider 50% mortality as the threshold value to discriminate resistance from susceptibility. The accessions with the best performance displayed SM values ranging from 0 to 44% (**Figure 2**).

In **bioassay #2**, 13 isolates induced severe symptoms in the susceptible controls, confirming their virulence. The *S. habrochaites* 'PI-127826' and 'PI-127827' accessions displayed resistant responses against different set of isolates employed in previous bioassays (Gonçalves-Pereira *et al*. 2023). The cultivars 'Ponderosa' and 'Santa Clara' were also included in the bioassay #2. It was possible to observe significant differences among accessions, among isolates and for the accession-isolate interactions (**Supplementary Table 3; Figure 3 and Figure 4**). The average test using the SM as a function of the isolate indicated 'PCp-167', 'PCp-182', and 'PCa-28' as the best performers (SM values above

50%). On the other hand, the isolates 'PCp-190', 'PCp-186', and 'PCp-188' displayed the lowest SM values (< 30%) (**Figure 3**). The cultivars 'Santa Clara' and 'Ponderosa' were susceptible and significantly different from *S. habrochaites* 'PI-127826' and 'PI-127827' (**Supplementary Table 4**). The difference among isolates detected by deviance analysis is due to 'Santa Clara' and 'Ponderosa', whereas *S. habrochaites* 'PI-127826' and 'PI-127827' accessions behaved similarly to each other when inoculated with different isolates (**Supplementary Table 4**), confirming their resistant responses as observed in a previous study using the isolates 'PCp183' and 'PCp192' (Gonçalves-Pereira *et al*., 2023).

In the **bioassay #3**, a selected group of ten isolates was employed to inoculate four *S. habrochaites* accessions ('PI-127826', 'PI-127827', 'LA 407', and 'WIR 7924') as well as five *S. lycopersicum* accessions ('CNPH 1143', 'Micro-Tom', 'Hawaii-7996', 'Santa Clara', and 'Ponderosa') that displayed sharp differential responses to *P. capsici* isolates in previous bioassays (Quesada-Ocampo *et al*., 2016; Petry *et al.* 2017a; Gonçalves-Pereira *et al.* 2023). It was once again possible to observe significant differences among accessions, among isolates and for accession-isolate interactions (**Supplementary Table 5**). Here, 50% seedling mortality was also established as the threshold value to discriminate resistance from susceptibility. The accessions 'PI-127826', 'PI-127827', 'Micro-Tom', 'CNPH 1143', and 'Hawaii-7996' showed the lowest SM values (**Supplementary Table 6**). Analyzing the SM values according to the isolates, 'PCp-182' and 'PCa-28' displayed significantly superior performances in comparison with the remaining isolates, inducing values above 50% (**Figure 5**). The *S. habrochaites* accessions 'PI-127826', 'PI-127827', 'LA 407', and 'WIR 7924' displayed different results when challenged with different isolates, while 'Hawaii-7996' was the single accession with interaction levels below threshold point for all isolates. *Solanum habrochaites* 'PI-127826' was above the threshold value only for the isolate 'PCa-28', whereas *S. habrochaites* 'PI-127827' was above the threshold value for the 'PCp-167' and 'PCp-192' isolates (**Figure 6**).

In a combined analysis (using 50% mortality as the threshold point) we were able to detect eight potential pathotypes within a subset of ten isolates (for which all accessions were challenged in one or more bioassays). These isolates were classified into pathotypes according to their unique interaction patterns with nine informative *Solanum* (*Lycopersicon*) accessions (**Table 3**), *viz*. 'Santa Clara', 'Hawaii-7996', 'CNPH 1143', 'Micro-Tom', 'Ponderosa', 'LA 407', 'WIR 7924', 'PI-127826', and 'PI-127827'. The Brazilian cultivar

‘Santa Clara’ displayed a “universal” susceptible reaction to all isolates used in all bioassays. Pathotype 03 was predominant, whereas the pathotype 05 displayed the largest virulence profile, being able to induce disease above the threshold point in five out of the nine accessions (**Table 3**). However, it is important to point out that most accessions showed a resistant response to pathotype 03, except for the ‘WIR 7924’ and the “universal” susceptible accession (‘Santa Clara’). In the other side, the pathotype 02 was able to induce disease only in the “universal” susceptible ‘Santa Clara’.

Discussion

Information concerning the genetic diversity of *P. capsici* populations (especially in relation to their virulence profiles) is an essential piece of information for breeding programs aiming for the development of phenotypically stable resistant cultivars (Barchenger *et al.* 2018a; Fan *et al.* 2022). Worldwide surveys of *P. capsici* isolates in the pathosystem involving *Capsicum* species are revealing subpopulations with remarkable phenotypic and genetic variability with many of them displaying sharp differences in their virulence profiles (Ares *et al*. 2005; Lee *et al.* 2010; Granke *et al*. 2012; Ribeiro and Bosland 2012; Castro-Rocha *et al.* 2016; Reyes-Tena *et al*. 2019a; Reyes-Tena *et al.* 2019b; Lee *et al.* 2021; Reyes-Tena *et al*. 2021; Saltos *et al.* 2022; Fan *et al*. 2022). A large number of potential physiological races (= pathotypes) has been proposed for *Capsicum*-infecting *P. capsici* isolates from North America (Ribeiro and Bosland 2012; Jiang et al. 2015; Barchenger *et al*. 2018b), from Korea (Lee *et al.* 2010), from Mexico (Reyes-Tena *et al.* 2019a), and from Spain (Silvar *et al.* 2006).

Significant accession-isolate interactions and distinct virulence profiles among *P. capsici* isolates have also been previously observed among *Solanum* (*Lycopersicon*) accessions (Quesada-Ocampo *et al.* 2016; Petry *et al*. 2017a; Gonçalves-Pereira *et al.* 2023), suggesting the putative presence of races/pathotypes. Here, we assessed the potential presence of distinct pathotypes and carried out a first attempt to establish a suitable set of differential host accessions in the *P. capsici*–*Solanum* (*Lycopersicon*) pathosystem. Seventeen virulent isolates were initially used to evaluate nine accessions that displayed sharp contrasting reactions to one or more isolates in previous bioassays. In the pathogen side, the ‘PCp-182’ isolate induced the highest SM values in the three bioassays and affected the highest number of accessions, whereas the other isolates displayed intermediate performance, inducing lower SM values restrict to a subgroup of bioassays. Therefore, ‘PCp-182’ (classified here as

pathotype 05) is the most suitable isolate for future inheritance studies since it displayed the broadest virulence profile. We observed here that isolates originally obtained from cucurbits (e.g., 'PCa-28') may induce similar levels of mortality in *Solanum* (*Lycopersicon*) accessions in comparison to those obtained from hosts also belonging to the Solanaceae family, diverging from the pattern reported by Quesada-Ocampo and Hausbeck (2010) using a panel of isolates from the USA. However, more studies are necessary to verify the effects on the virulence profile according to the original plant host of the isolates.

Significant accession-isolate interactions were observed in all assays, indicating that disease onset and development depends upon the interaction between a specific virulent phenotype and a *Solanum* (*Lycopersicon*) accession displaying a pathotype-specific compatibility. On the host side, accessions resistant to one isolate but highly susceptible to another isolate were observed in all bioassays. For example, in bioassay #1, the cultivar 'Ponderosa' was resistant to isolates 'PCa-31', 'PCp-115', 'PCp-188', 'PCp190', 'PCp191', 'PCp192', and 'PCt38', but was highly susceptible to isolates 'PCa-28', 'PCp-153', 'PCp-167', 'PCp-182', 'PCp-183', 'PCp-189', 'PCa-29', and 'PCp-187'. Using the 50% mortality as the threshold value for the host-pathogen interactions, we were able to detect eight potential pathotypes among a subset of ten isolates according to their unique interaction patterns with nine informative *Solanum* (*Lycopersicon*) accessions (viz. 'Santa Clara', 'Hawaii-7996', 'CNPH 1143', 'Micro-Tom', 'Ponderosa', 'LA 407', 'WIR 7924', 'PI-127826', and 'PI-127827'). Even though distinct virulence profiles were observed among these isolates, none of them could simultaneously affect all identified sources of resistance, which is relevant and practical information for tomato genetic improvement programs.

Based upon its overall genetic and phenotypic diversity as well as its spatio-temporal dispersion throughout the world (Barchenger *et al.* 2018a), the most likely center of origin of *P. capsici* encompasses South of the United States, Meso-America, and the Pacific Coast and Andean region of South America. In relation to the genus *Solanum* (section *Lycopersicon*), the center of origin of the 13 related species encompasses the Coastal and Andean regions of Ecuador (including Galapagos Islands), Peru, Colombia, Bolivia, and Chile (Bauchet and Cause, 2012). The initial center of tomato domestication was Peru and the secondary domestication centers are more likely sites in South Mexico and Guatemala (Bauchet and Cause, 2012). These natural concurrent areas of incidence of both *Solanum* hosts and pathogen isolates may allowed continuous co-evolutionary interactions, which corroborates

for the natural emergence of a plethora of different virulence phenotypes. The co-evolutionary interactions of *P. capsici* and *Solanum* (section *Lycopersicon*) hosts in these multiple and fragmented geographic areas may allowed the emergence of isolates with broad virulence profile but, in the other hand, may also allowed the unintentional human selection of domesticated tomatoes with both broad-spectrum as well as pathotype-specific resistances (Zentmyer, 1988).

The long-distance dispersal mechanisms of *P. capsici* are not fully elucidated (Lamour *et al.* 2012). In Brazil, *P. capsici* is possibly an introduced pathogen and, therefore, lower natural levels of genetic divergence are expected. However, the two distinct mating types (A1 and A2) are present in Brazil (Rêgo and Reifschneider 1982; Paz-Lima 2006; Petry *et al*. 2017b) and they may be playing a very important role in generating diversity in the virulence phenotypes of field isolates. Here, eight pathotypes were identified in a panel of only ten isolates, demonstrating the dynamic nature of this pathogen. Similar results were also observed by Reyes-Tena *et al.* (2019a) in studies of virulence phenotypes of infective isolates of hot peppers from Michoacán (Mexico), where ten distinct virulence phenotypes were detected in ten field-collected isolates. Jiang et al. (2015) observed that from 13 *P. capsici* isolates collected in chili pepper fields in New Mexico (USA), 12 were identified as new races. On the other hand, only seven distinct pathotypes were identified among 37 *Capsicum*-infecting isolates from Eastern China (Sun *et al.* 2008).

In this scenario of wide virulence profiles, precise methods of pathotype detection in *P. capsici* populations are necessary. However, the lack of a standard race/pathotype classification system with a standard set of differential accessions makes difficult monitoring and comparing the worldwide virulence diversity of this pathogen (Barchenger *et al.* 2019). In *Capsicum* species, recombinant inbred strains (RILs) are often used as differentials (Barchenger *et al.* 2018b; Reyes-Tena *et al*. 2019; Vogel *et al.* 2021). RILs are homozygous genotypes which seeds can be permanently multiplied without significant segregation. In *Solanum* (*Lycopersicon*) it is relatively easy to obtain highly homozygous inbred lines for use as differential accessions, even though it might be more problematic with allogamous wild species such as *S. habrochaites* (Markova *et al.* 2016). The precise determination of potential races/pathotypes will also require the standardization of other major relevant factors for this type of bioassays including, inoculation methodology, plant age, as well as the most suitable

environmental conditions for both host and pathogen (Reifschneider *et al.* 1986; Barchenger *et al*. 2018a, Nawaz *et al.* 2018).

In the present study, a relatively large number of isolates displayed a drastic loss of infectivity during their *in vitro* maintenance. Only 17 out of 31 the isolates were able to infect the susceptible control *C. annuum* 'Tico'. Somewhat surprisingly, the loss of the ability to induce disease was observed with the isolate 'PCpe-09', which induced severe symptoms in many tomato accessions in recent bioassays carried out by Petry *et al*. (2017a). Similar loss of infectivity was observed here for the isolates 'PCp-106', 'PCp-126' and 'PCt-26', which were previously able to induce severe symptoms in *C. annuum* lines (Ribeiro and Bosland, 2012). This type of phenotypic change in the pathogenicity patterns might be consequence of loss of heterozygosity, a hypothesis put forward by Vogel *et al.* (2021) in which spontaneous chromosome loss and duplication leads to phenotypic variability, even within clonal lineages of *P. capsici*. Fast and drastic loss of pathogenicity in isolates with less than one-year after collection was also reported by Reyes-Tena *et al.* (2019a) in two *Capsicum*-infecting *P. capsici* isolates from Mexico.

The *S. habrochaites* 'LA 407' accession was the first promising source of resistance to *P. capsici* (Quesada-Ocampo and Hausbeck, 2010; Quesada-Ocampo *et al.* 2016). Our results are confirming three new *S. habrochaites* accessions (viz. 'WIR 7924', PI-127826', 'PI-127827') with immune-like resistance to a wide array of *P. capsici* isolates. These three new accessions plus *S. habrochaites* 'LA 407' displayed informative differential (resistant/susceptible) responses to a specific set of isolates. These four *S. habrochaites* accessions are, therefore, potential candidates as differential lines for pathotype determination assays. In the present study, the accessions *S. habrochaites* 'PI-127826' and 'PI-127827' displayed a larger spectrum of immune-like resistance response to *P. capsici* isolates in comparison with *S. habrochaites* 'LA 407'. This is an interesting result since 'LA 407' is thus far the best source of resistance to *P. capsici* isolates from North America (Quesada-Ocampo and Hausbeck, 2010; Quesada-Ocampo *et al.* 2016). The *S. habrochaites* 'PI-127826' and 'PI-127827' accessions reported here with large-spectrum resistance may be immediately incorporated in tomato breeding programs aiming for the development of rootstocks for use in areas already infested with this pathogen (Reis *et al.* 2021).

The inbred line *S. lycopersicum* 'Hawaii-7996' has demonstrating a superior and stable large-spectrum resistant performance to the majority of the *P. capsici* isolates in distinct bioassays carried out in Brazil (Petry *et al.* 2017a; Goncalves-Pereira *et al.* 2023; present study). Interestingly, 'Hawaii-7996' displayed heterogenous/unstable reactions only to the 'PCp-191' isolate, being susceptible in bioassay #1 as well as in a different bioassay reported by Gonçalves-Pereira *et al.* (2023). This unstable response (with the presence of a quite variable number of susceptible plants) has been observed even in the Mexican landrace 'CM334', which is the best source of resistance reported thus far in *Capsicum* germplasm (Retes-Manjarrez *et al.* 2020). This kind of unstable reaction can be explained by a potential oligogenic or polygenic control of the resistance (Reifschneider *et al.* 1992), which phenotypic expression might be more strongly affected by environmental factors.

Here, we found that the tomato cultivar 'Santa Clara' might function as the universal susceptible line since it displayed 100% mortality to all isolates. 'Santa Clara' is, therefore, an outstanding accession to serve as the susceptible parental line in inheritance and mapping studies, minimizing "phenotyping noises" due to disease-escaped plants in segregating populations. This is an important information since other susceptible tomato lines used in genetic studies did not show similar levels of susceptible reaction. *Solanum* (*Lycopersicon*) highly susceptible accessions allowing for 100% mortality are, in fact, quite difficult to be detected (Quesada-Ocampo *et al*. 2016; Goncalves-Pereira *et al.* 2023). One example is the cultivar 'Hunt100', which has been employed as a susceptible standard in mapping studies. This cultivar displayed mortality ranging from 54.7 to 73.6%, according to the isolate (Quesada-Ocampo *et al.* 2016).

The *S. lycopersicum* 'Hawaii-7996', followed by *S. habrochaites* 'PI-127826' and 'PI-127827', were the best sources of large-spectrum resistance, displaying superior performance to a wide array of Neotropical isolates for employment as parental accessions in resistance breeding programs. These accessions are also suggested as potential differential lines in pathotype determination assays in this pathosystem. Future inheritance and mapping studies of these pathotype-specific resistances in each *Solanum* (*Lycopersicon*) accession will facilitate their incorporation into commercial tomato cultivars and will allow a more precise determination of the number of pathotypes among Neotropical *P. capsici* isolates, according to their ability of 'breaking-down' a specific set of genes in this germplasm.

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Table 1 – Nine *Solanum* (section *Lycopersicon*) accessions evaluated for their reaction to *Phytophthora capsici* isolates in three controlled greenhouse bioassays reported in the present study.

Accession code	***Solanum* (section *Lycopersicon*) species and cultivars**
'H-7996'	*Solanum lycopersicum* 'Hawaii 7996' – J.W. Scott (Univ. Florida)
'CNPH 1143'	*Solanum lycopersicum* 'Tetéia'
'CNPH 0420'	*Solanum habrochaites* 'PI 127826'
'CNPH 0421'	*Solanum habrochaites* 'PI 127827'
'CNPH 1286'	*Solanum habrochaites* 'PI 251304' = 'LA 407' (Selection from Ecuador)
'CNPH 0929'	*Solanum habrochaites* 'WIR 7924'
'CNPH 1284'	*Solanum lycopersicum* 'Micro-Tom'
'Ponderosa'	*Solanum lycopersicum* (Heirloom cultivar)
'Santa Clara'	*Solanum lycopersicum* (commercial Brazilian cultivar / IAC-SP)

Table 2 – Complete list of 30 *Phytopthora capsici* isolates obtained from different hosts and regions of Brazil and employed for pathogenicity bioassays. Only a subgroup of 17 isolates was virulent (= able to induce disease) in the susceptible control *Capsicum annuum* 'Tico'. This subgroup of 17 viruent isolates was then employed in the bioassays reported in the present study.

Code	Isolate designation	Collection site	Year	Original host	Virulent / Avirulent
01	'PCa28'	Nova Friburgo–RJ	2008	*Cucurbita pepo*	**Virulent**
02	'PCa29'	Nova Friburgo–RJ	2008	*Cucurbita pepo*	**Virulent**
03	'PCa31'	Uberaba–MG	2006	*Cucurbita moschata*	**Virulent**
04	'PCp106'	Barbacena–MG	2006	*Capsicum annuum*	Avirulent
05	'PCp109'	Morrinhos–GO	2006	*Capsicum annuum*	Avirulent
06	'PCp115'	Abadiânia–GO	2007	*Capsicum baccatum*	**Virulent**
07	'PCp117'	Nova Friburgo–RJ	2008	*Capsicum annuum*	Avirulent
08	'PCp120'	Monte Carmelo–MG	2008	*Capsicum annuum*	Avirulent
09	'PCp126'	Gama–DF	2008	*Capsicum annuum*	Avirulent
10	'PCp139'	Nova Friburgo–RJ	2008	*Capsicum annuum*	Avirulent
11	'PCp142'	Guaraciaba–CE	2009	*Capsicum annuum*	Avirulent
12	'PCp148'	Guaraciaba–CE	2009	*Capsicum annuum*	Avirulent
13	'PCp153'	Ouvidor–GO	2009	*Capsicum annuum*	**Virulent**
14	'PCp156'	Brazlândia–DF	2009	*Capsicum annuum*	Avirulent
15	'PCp159'	Bragança Paulista–SP	NA*	*Capsicum* sp.	**Virulent**
16	'PCp167'	Vagem Bonita–DF	NA	*Capsicum annuum*	**Virulent**
17	'PCp169'	Vargem Bonita–DF	NA	*Capsicum annuum*	Avirulent
18	'PCp182'	Ceilândia–DF	NA	*Capsicum chinense*	**Virulent**
19	'PCp183'	Ceilândia–DF	NA	*Capsicum chinense*	**Virulent**
20	'PCp186'	Brasília–DF	NA	*Capsicum chinense*	**Virulent**
21	'PCp187'	Brasília–DF	NA	*Capsicum chinense*	**Virulent**
22	'PCp188'	Brasília–DF	NA	*Capsicum chinense*	**Virulent**
23	'PCp189'	Brasília–DF	NA	*Capsicum chinense*	**Virulent**
24	'PCp190'	Brasília–DF	NA	*Capsicum chinense*	**Virulent**
25	'PCp191'	Brasília–DF	NA	*Capsicum chinense*	**Virulent**
26	'PCp192'	Santa Maria–DF	2022	*Capsicum chinense*	**Virulent**
27	'PCpe09'	Viçosa–CE	2009	*Cucumis sativus*	Avirulent
28	'PCt26'	Morrinhos–GO	2006	*Solanum lycopersicum*	Avirulent
29	'PCt38'	Brasília–DF	2010	*Solanum lycopersicum*	**Virulent**
30	'PCVg'	Brasília–DF	2007	*Phaseoulus vulgaris*	Avirulent

*NA= information not available.

Table 3 – Eight potential pathotypes detected in a sample of ten *Phytopthora capsici* isolates according to their patterns of interaction with nine informative *Solanum* (*Lycopersicon*) accessions. This subset of isolates was selected because they were used to challenge (in one or more bioassays) all differential host accessions: *S. habrochaites* 'PI 127826', 'PI 127827', 'LA 407', and 'WIR 7924', and *S. lycopersicum* 'CNPH 1143', 'Micro-Tom' (MT), 'Hawaii-7996' (H-7996), and 'Ponderosa' (Pond). The cultivar 'Santa Clara' was employed as the "universal" susceptible line since it displayed 100% mortality (1.00) for all isolates. These resistant (R) and susceptible (S) reactions were statistically defined based upon the cutoff/threshold level of 50% mortality rate.

Isolate	'WIR 7924'	CNPH 1143	MT	H-7996	LA 407	PI-127826	PI-127827	Pond	Pathotype
PCa28	**0.78 S**	0.00 R	0.44 R	0.00 R	**0.67 S**	**0.56 S**	0.44 R	**0.67 S**	**01**
PCa31	0.00 R	0.33 R	0.33 R	0.11 R	0.00 R	0.00 R	0.00 R	0.33 R	**02**
PCp115	**0.56 S**	0.11 R	0.22 R	0.33 R	0.00 R	0.33 R	0.33 R	0.33 R	**03**
PCp167	0.11 R	0.33 R	0.11 R	0.00 R	0.00 R	0.44 R	**0.56 S**	**0.67 S**	**04**
PCp182	**0.67 S**	**0.78 S**	**0.89 S**	0.44 R	**0.67 S**	0.33 R	0.00 R	**1.00 S**	**05**
PCp183	0.11 R	0.44 R	0.00 R	0.00 R	**0.89 S**	0.33 R	0.00 R	**0.67 S**	**06**
PCp187	0.22 R	0.00 R	0.00 R	0.00 R	0.00 R	0.00 R	0.00 R	**0.67 S**	**07**
PCp188	**0.78 S**	0.22 R	0.44 R	0.44 R	0.00 R	0.00 R	0.00 R	0.00 R	**03**
PCp190	**0.56 S**	0.33 R	0.22 R	0.33 R	0.33 R	0.00 R	0.22 R	0.00 R	**03**
PCp192	0.00 R	**0.56 S**	0.22 R	0.00 R	**0.89 S**	0.22 R	**0.89 S**	0.33 R	**08**

Supplementary Table 1 – Deviance analysis for ability of 14 *Phytophthora capsici* isolates to induce seedling mortality in six *Solanum* (section *Lycopersicon*) accessions in the **bioassay #1**.

	Degree of freedom (DF)	Deviance	Residual DF	Residual Deviance	Pr(>Chi)	
Null			755.00	1034.23		
Accession	5	265.12	750.00	769.11	0.00	***
Isolate	13	70.91	737.00	698.21	0.00	***
Accession-isolate	65	187.15	672.00	511.06	0.00	***

*** Significantly different at the 0.001 level for Chi-square tabulated.

Supplementary Table 2 – The overall estimated mortality rates of six *Solanum* (section *Lycopersicon*) accessions induced by 14 *Phytophthora capsici* isolates in the **bioassay #1**.

Accession	**Seedling mortality**	**Reaction group***
'Santa Clara'	1.00	A
'Ponderosa'	0.44	B
'CNPH 1143'	0.33	C
'Micro-Tom'	0.33	C
'WIR 7924'	0.31	C
'Hawaii-7996'	0.18	D

* Means in the same column, followed by a common letter, do not differ significantly according to Scott Knott's test (P = 0.05).

Supplementary Table 3 – Deviance analysis for the ability of 13 *Phytophthora capsici* isolates to induce seedling mortality in four *Solanum* (section *Lycopersicon*) accessions in the **bioassay #2**.

	Degree of freedom (DF)	Deviance	Residual DF	Residual Deviance	Pr(>Chi)	
Null			727.00	984.42		
Accession	3	482.94	724.00	501.48	0.00	***
Isolate	12	68.22	712.00	433.26	0.00	***
Accession-isolate	36	60.94	676.00	372.33	0.01	**

'***' '**' Significantly different at the 0.001 and 0.01 level for Chi-square tabulated.

Supplementary Table 4 – The overall estimated mortality rates induced by 13 *Phytophthora capsici* isolates in four *Solanum* (section *Lycopersicon*) in the **bioassay #2**.

Accession	**Seedling mortality**	**Reaction group***
'Santa Clara'	1.00	A
'Ponderosa'	0.41	B
'PI 127826'	0.13	C
'PI 127827'	0.10	C

* Means in the same column, followed by a common letter, do not differ significantly according to Scott Knott's test (P = 0.05).

Supplementary Table 5 – Deviance analysis for the ability of ten *Phytophthora capsici* isolates to induce seedling mortality in nine *Solanum* (section *Lycopersicon*) accessions in the **bioassay #3**.

	Degree of freedom (DF)	Deviance	Residual DF	Residual Deviance	Pr(>Chi)	
Null			810.00	1076.94		
Accession	8	220.02	802.00	856.91	0.00	***
Isolate	9	66.41	793.00	790.50	0.00	***
Accession-isolate	72	249.66	721.00	540.84	0.00	***

'***Significantly different at the 0.001 level for Chi-square tabulated.

Supplementary Table 6 – The overall estimated mortality rates induced by ten *Phytophthora capsici* isolates in four *Solanum* (section *Lycopersicon*) in the **bioassay #3**.

Accession	Seedling mortality	Reaction group*
'Santa Clara'	1.00	A
'Ponderosa'	0.47	B
'WIR 7924'	0.38	B
'LA 407'	0.34	B
'CNPH 1143'	0.31	C
'Micro-Tom'	0.29	C
'PI-127827'	0.24	C
'PI-127826'	0.22	C
'Hawaii-7996'	0.17	C

* Means in the same column, followed by a common letter, do not differ significantly according to Scott Knott's test (P = 0.05).

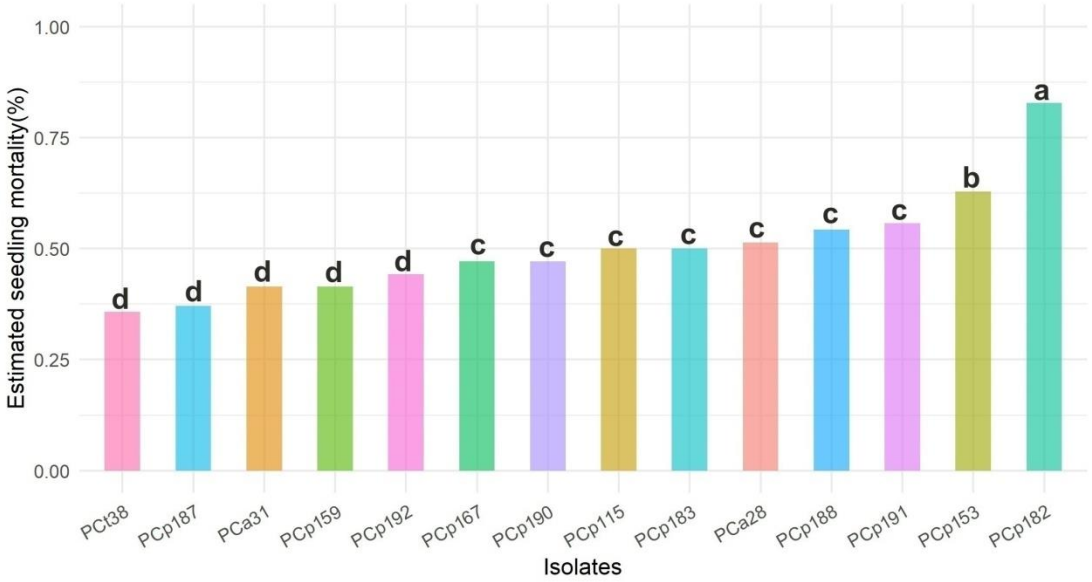

Fig. 1 – Estimated seedling mortality of one *Solanum* (*Lycopersicon*) accession induced by 14 *Phytophthora capsici* isolates in the **bioassay #1**. Means in the same column, followed by a common letter, do not differ significantly according to Scott Knott's test (P = 0.05).

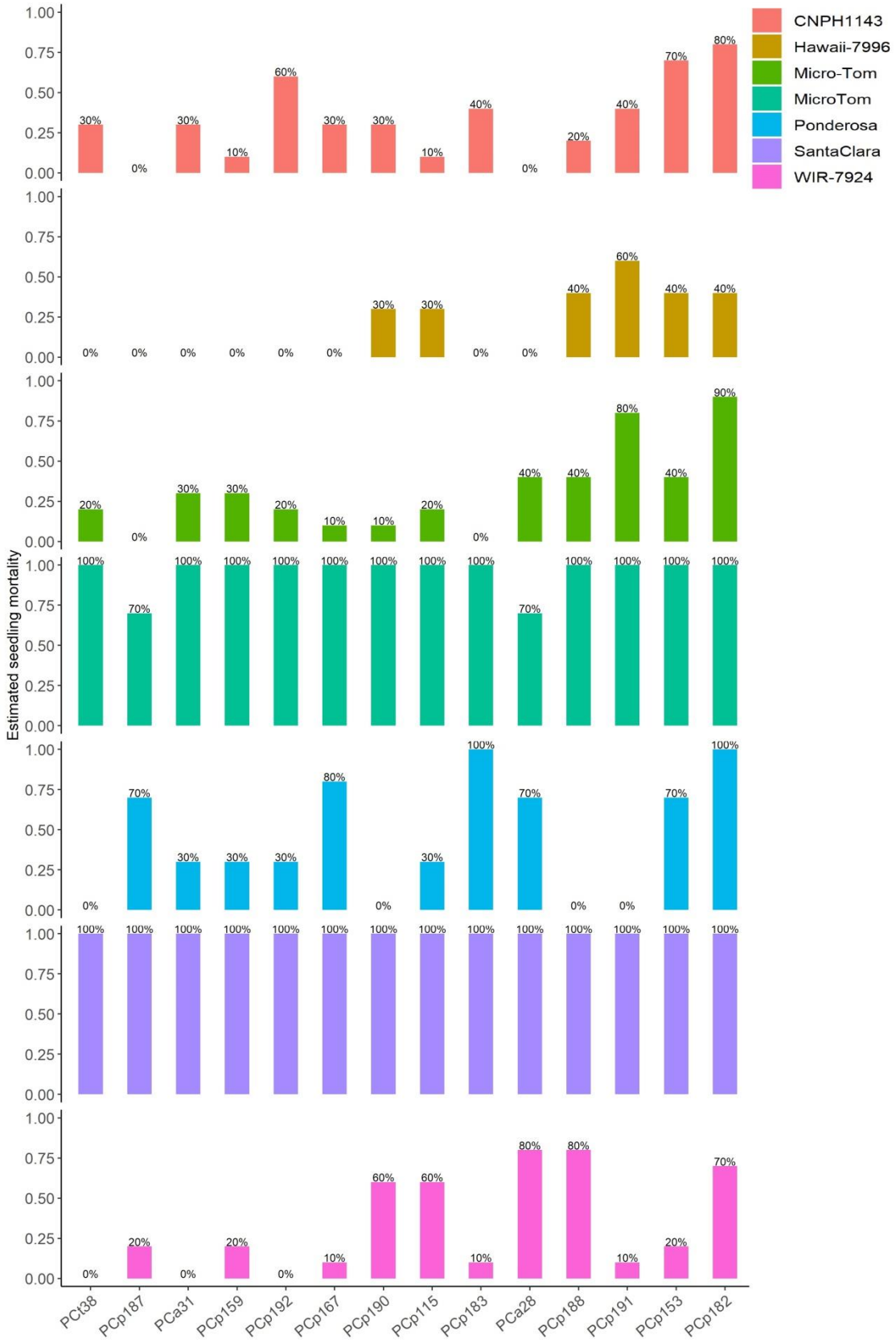

Fig. 2 – Estimated seedling mortality of *Solanum habrochaites* 'CNPH 0929' (= 'WIR 7924') and *S. lycopersicum* 'Ponderosa'; 'Santa Clara'; 'Hawaii-7996' (= 'H-7996'); 'CNPH 1143', and Micro-Tom (= 'CNPH 1284') in response to the inoculation with a panel of 14 *Phytophthora capsici* in the **bioassay # 1**.

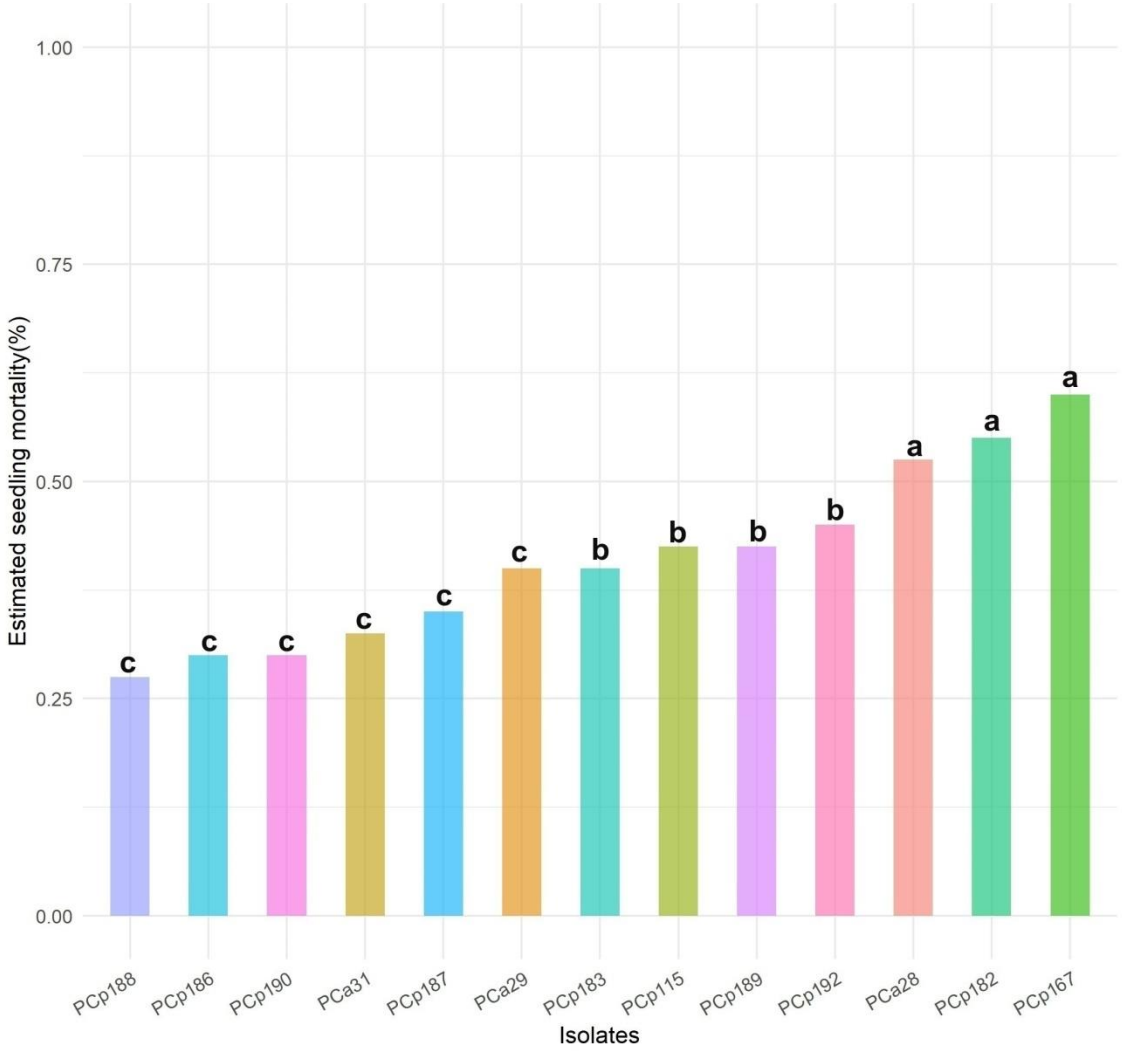

Fig. 3 – Estimated seedling mortality of two *Solanum* (*Lycopersicon*) accessions induced by 13 *Phytophthora capsici* isolates the **bioassay #2**. Means in the same column, followed by a common letter, do not differ significantly according to Scott Knott's test (P = 0.05).

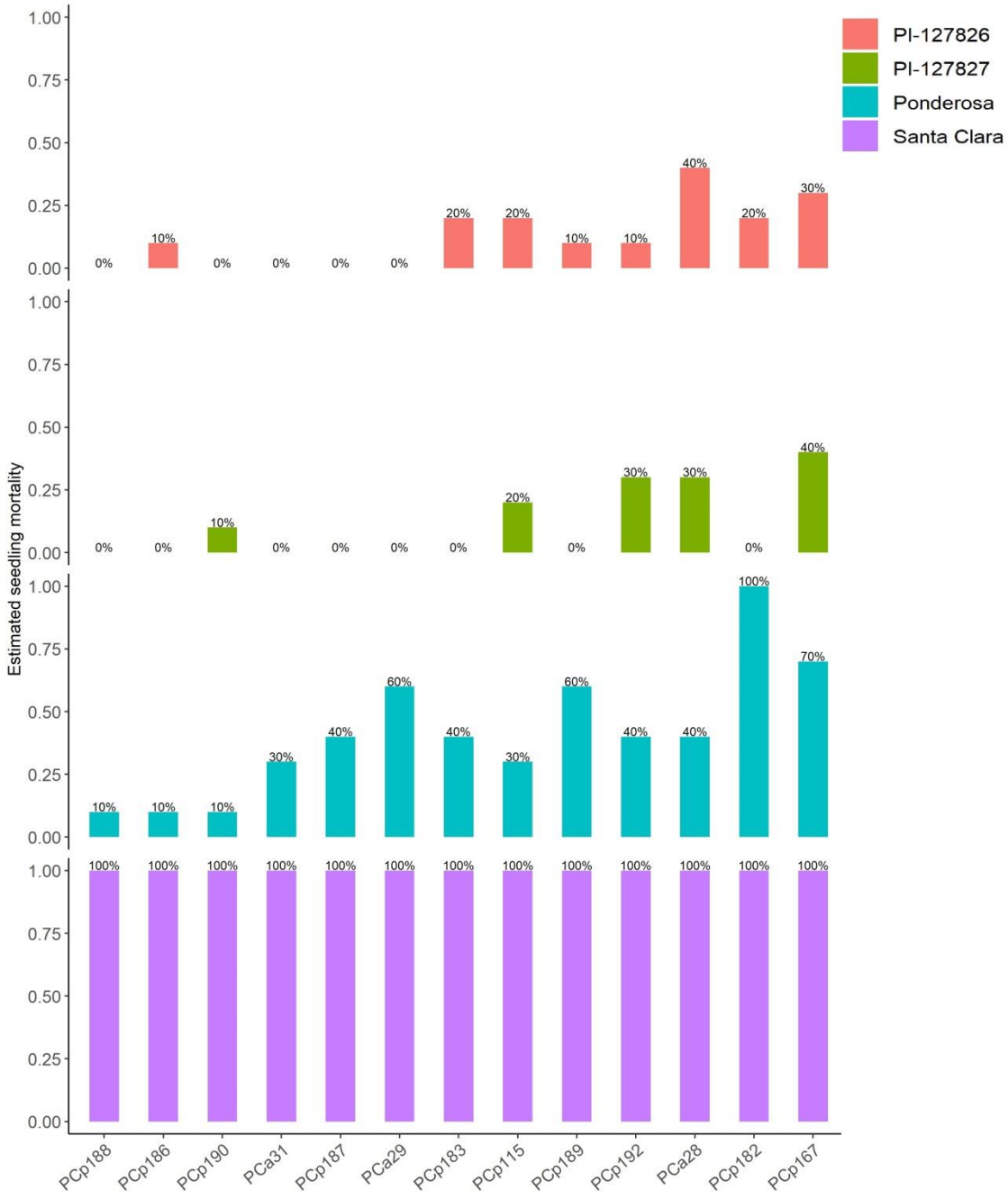

Fig. 4 – Seedling mortality rates of two *Solanum habrochaites* accessions ('PI-127826' and 'PI-127827') and two *Solanum lycopersicum* accessions ('Ponderosa' and 'Santa Clara') in response to the inoculation with 13 *Phytophthora capsici* isolates in the **bioassay # 2**.

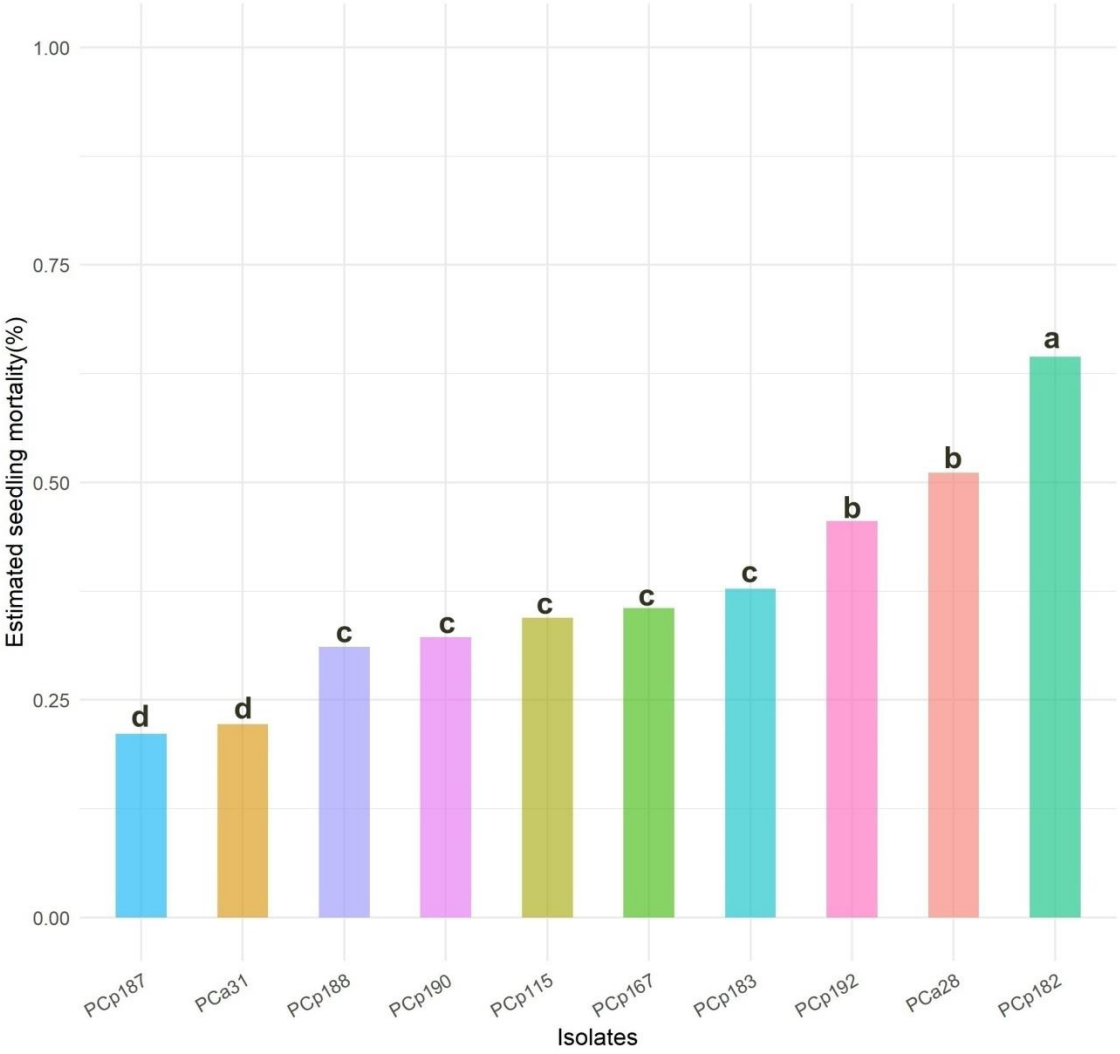

Fig. 5 – Estimated seedling mortality induced by ten *Phytophthora capsici* isolates in nine *Solanum* (section *Lycopersicon*) accessions in the **bioassay #3**.

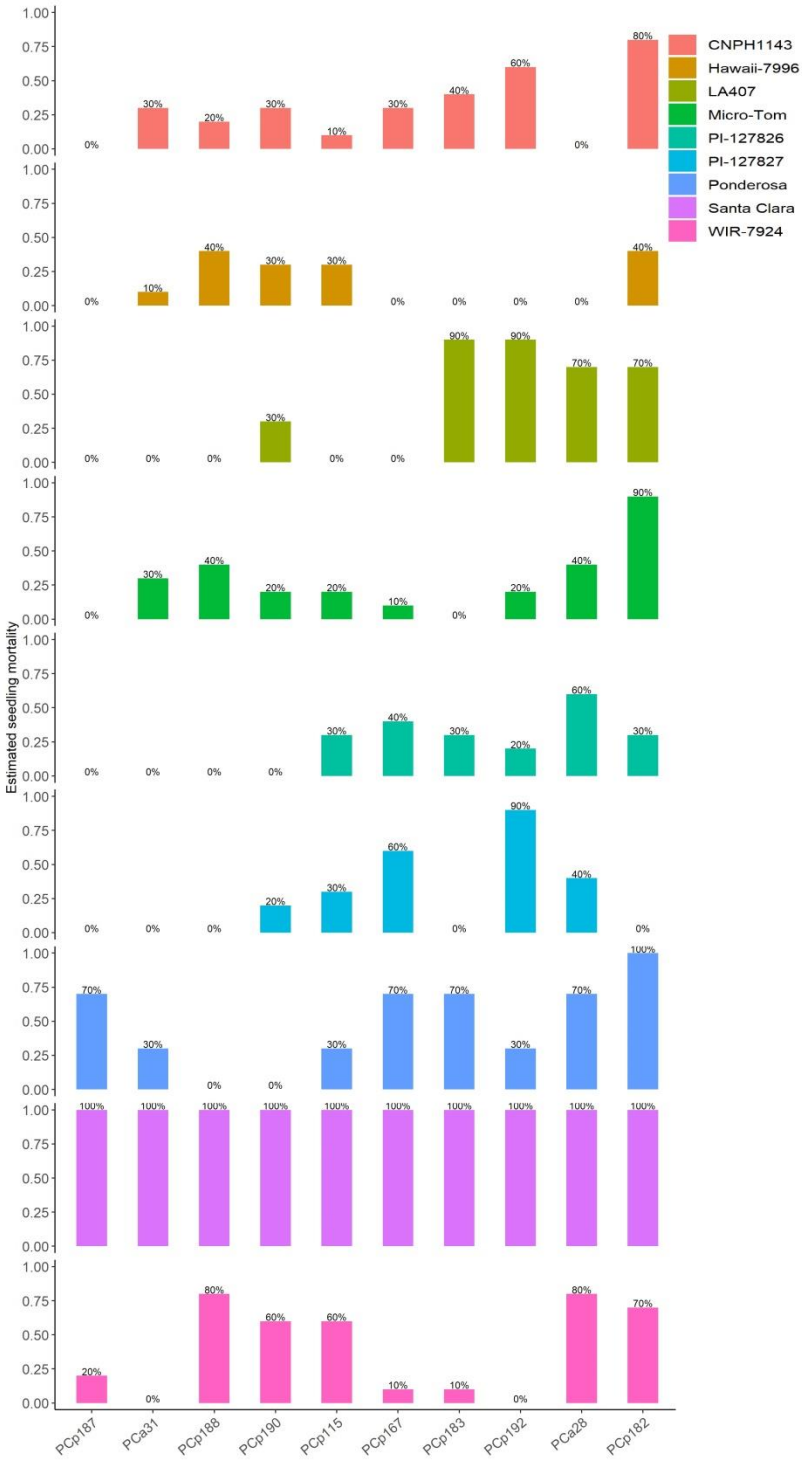

Fig. 6 – Estimated seedling mortality of four *Solanum habrochaites* accessions 'PI-127826', 'PI-127827', 'LA 407', and 'CNPH 0929' (= 'WIR 7924') and four *Solanum lycopersicum* accessions 'Ponderosa', 'Santa Clara', 'Hawaii-7996' (= 'H-7996'), 'CNPH 1143', and Micro-Tom (= 'CNPH 1284') in response to the inoculation with ten *Phytophthora capsici* isolates in the **bioassay #3**.

CAPÍTULO V

A duplicate dominant epistatic model for resistance to *Phytopthora capsici* in *Solanum habrochaites* 'PI 127827'

A duplicate dominant epistatic model for resistance to *Phytopthora capsici* in *Solanum habrochaites* 'PI 127827'

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Abstract

Efficient cultural and chemical management of *Phytophthora capsici* has a very problematic implementation in tomatoes (*Solanum lycopersicum*) and other hosts. In this scenario, commercial cultivars with large-spectrum genetic resistance would be the most sustainable strategy. Thus far, *S. habrochaites* 'PI 127827' is one of the best sources of resistance to a wide array of Brazilian isolates. However, inheritance studies of resistance to *P. capsici* in this wild tomato species are yet scarce. Here, biparental crosses and controlled inoculation assays were carried out to determine the genetic basis of *P. capsici* resistance in 'PI 127827'. Populations were developed using as susceptible female parent the heirloom cultivar 'Ponderosa'. Inoculation was carried at seedling stage by depositing 3 mL of a suspension (2×10^4 zoospores/mL) around the crown area of the contrasting parents and in plants of the F_1 and F_2 generations. Evaluation was done when 100% of the 'Ponderosa' plants were collapsed. 'PI 127827' and F_1 plants displayed a very strong resistant reaction, indicating a dominant control with high levels of penetrance. The F_2 generation displayed a segregation of 226 resistant to 24 susceptible plants. The chi-square goodness-of-fit test for F_2 segregating frequency best conformed to the 15:1 ratio, indicating the involvement of a duplicate dominant epistatic model for resistance in 'PI 127827'. Under this model, the resistant phenotype expression needs only one of the two dominant genes in heterozygous condition. We tentatively named these two loci as *Phythphthora capsici resistance* 1 (*Phcr*1) and 2 (*Phcr*2). This relatively simple genetic control will facilitate the introgression of this trait into elite tomato lines.

Keywords: Breeding, soil-borne pathogens, oospores, sporangia, tomato.

Introduction

Phytophthora capsici Leonian is a very destructive soil-borne oomycete of a multitude of vegetable and field crops (Reis *et al.* 2018; Saltos *et al.* 2022). *Phytophthora capsici* has been reported as a pathogen of tomato (*Solanum lycopersicum* L.) since the late 1930s (Kreutzer *et al.* 1940), inducing different disease syndromes such as damping-off, root and/or collar rot in fresh-market tomatoes as well as fruit rot in processing tomato crops (Parada-Rojas *et al.* 2021). The chemical and cultural management of *P. capsici*-induced diseases is of difficult implementation due its large host range of the pathogen; its tolerance to increasing doses of the major active ingredients of pesticides such as metalaxyl and fluazinam (Café-Filho and Ristaino, 2008; Siegenthaler and Hansen, 2021; Quispe-Quispe *et al*. 2022); its long-term survival ability in host-free soils as oospores, and its efficient polycyclic field dispersion by irrigation systems (Café-Filho and Duniway, 1995; Café-Filho and Duniway, 1996; Café-Filho *et al.* 1995). Alternative management strategies employing biocontrol agents are yet incipient (Wang *et al.* 2022; Volynchikova and Kim, 2022; Li *et al*. 2022). To further aggravate the scenario, there is also an extensive variation in the virulence profile of the pathogen isolates with sharp contrasting reactions among host accessions (Sanogo *et al.* 2022; Mohammadbagheri *et al*. 2022).

In recent studies focused on the *P. capsici–Solanum* (*Lycopersicon*) pathosystem, eight potential pathotypes were identified in field-collected isolates from distinct hosts and geographical locations in Brazil (Gonçalves-Pereira *et al.* 2023b). In this scenario, the deployment of commercial cultivars with large-spectrum genetic resistance to distinct pathotypes would be the most economically viable and ecologically sustainable strategy (Saltos *et al.* 2021). However, there are few promising sources of large-spectrum resistance to *P. capsici* identified and many of them belong to a subgroup of wild *Solanum* (section *Lycopersicon*) accessions (Quesada-Ocampo and Hausbeck, 2010; Quesada-Ocampo *et al*. 2016; Petry *et al.* 2017; Gonçalves-Pereira e*t al*. 2023a).

The wild species *S. habrochaites* Knapp and Spooner (former *Lycopersicon hirsutum* Dunal) was the first promising source of resistance to *P. capsici* detected in tomato accessions (Quesada-Ocampo and Hausbeck, 2010; Quesada-Ocampo *et al*. 2016). This wild species is also an outstanding source of resistance to a wide array of biotic and abiotic stresses (Miao *et*

al. 2022). Previous works are confirming the presence of useful genetic resistance factors against *P. capsici* in *S. habrochaites* accessions, which can be potentially transferred into commercial tomato cultivars via conventional breeding. *Solanum habrochaites* 'LA 407' is the best source of resistance to *P. capsici* isolates reported thus far (Quesada-Ocampo and Hausbeck, 2010; Quesada-Ocampo *et al*. 2016). However, the accession *S. habrochaites* 'PI 127827' displayed superior levels of immune-like resistance to a wide array of *P. capsici* isolates (Gonçalves-Pereira *et al.* 2023a; Gonçalves-Pereira *et al.* 2023b) when compared with *S. habrochaites* 'LA 407'.

From the breeding standpoint, inheritance studies of the *P. capsici* resistance will facilitate the incorporation of this trait into improved host cultivars. However, inheritance studies of resistance to *P. capsici* in *Solanum* (section *Lycopersicon*) accessions are yet incipient and little explored (Petry *et al.* 2017; Gonçalves-Pereira *et al.* 2023a; Gonçalves-Pereira *et al.* 2023b). Here, biparental crosses and controlled inoculation assays of segregating populations were carried out to determine the genetic basis of resistance to this pathogen in *S. habrochaites* 'PI 127827', which is a crucial information towards the development of phenotypically stable and large-spectrum resistant cultivars.

Material and Methods

Contrasting parental lines and controlled crosses – Two contrasting *Solanum* (section *Lycopersicon*) accessions were used as parental materials to investigate the genetic basis of resistance to *P. capsici* in the present study. As the resistant parent was employed *S. habrochaites* 'PI 127827' and the heirloom cultivar 'Ponderosa' was used as the highly susceptible parent. The corresponding F_1 and F_2 populations were developed using 'Ponderosa' only as the female parent and *S. habrochaites* 'PI 127827' as male parent (= pollen donor). Reciprocal crosses were not done due to unilateral incompatibility when using *S. habrochaites* as female parent (Broz *et al*. 2021).

Inoculum preparation – The highly virulent Brazilian *P. capsici* 'PCp-182' isolate was used for evaluation of the contrasting parents ('Ponderosa' and *S. habrochaites* 'PI 127827') and the corresponding F_1 and F_2 populations. The pure *P. capsici* cultures were transferred to Petri dishes containing 20% V8 agar medium (100 mL V8 juice, 1.5 g calcium carbonate, 10 g

agar, and 400 mL distilled water). Cultures were kept in growth chambers for 3 days in the dark and for 3 to 5 days under continuous light (25 ± 2 °C) to induce abundant sporangia production. Then, 20 mL of sterile distilled water was added to each plate and the plates were transferred to 6 °C for one hour, followed by 30 minutes at 25°C to induce zoospores production. The zoospores suspension was homogenized and filtered through sterile gauze. The estimation of the zoospore concentration was made with the aid of the Neubauer's chamber. When the young seedlings were displaying two pairs of fully open true leaves (25 days after sowing), they were removed from the tray cells and transplanted onto 1.5 L plastic pots (three plants per pot) containing sterile substrate. These tomato seedlings were then inoculated via deposition in the crown area of 3 mL of a suspension of each isolate (separately) adjusted to 2×10^4 zoospores mL^{-1}.

Evaluation and statistical analysis – The disease incidence (= number of plants with either severe symptoms or entirely collapsed) of all plants across all populations was evaluated when the 'Ponderosa' seedlings were 100% affected. The evaluation of was performed based upon the incidence of the disease. Segregation ratios of resistant (R) and susceptible (S) disease reactions were subjected to chi-square test to find out the goodness of fit to different classical Mendelian ratios with the assumed phenotypic ratios of F_2. Based on the phenotypic data obtained in the F_2 population, hypotheses of expected segregation were tested for one, two, and three genes, considering a significance level of 5%. Phenotypic ratios were tested for 22 hypotheses: one gene (3:1 and 1:3), two genes (15:1, 13:3, 7:9, 9:7, 3:13, and 1:15) and three genes (63:1, 61:3, 55:9, 37:27, 27:37, 9:55, 3:61, 1:63, 57:7, 51:13, 49:15, 43:21, 25:39, and 19:45). The statistical analyses were performed using the software package GENES (Cruz, 2016).

Results

All 25 inoculated plants of *Solanum habrochaites* 'PI 127827' were free of symptoms at 21 days after inoculation (DAI), whereas all 25 plants of 'Ponderosa' displayed severe crown-rot after inoculation with the *P. capsici* 'PCp-182' (**Figure 1 and Table 1**). The F_1 progeny displayed a resistant response similarly to the resistant parent with the majority (68) of the 70 inoculated plants remaining asymptomatic. This resistant reaction of the F_1 generation indicated a dominant control with high (but not complete) levels of penetrance. This resistant reaction observed in the unilateral F1 generation allowed us to exclude the action of

cytoplasmatic factors in the expression of the resistance since reciprocal crosses were not done due to unilateral incompatibility when using *S. habrochaites* as female parent.

The F_2 generation displayed a segregation of 226 resistant to 24 susceptible plants. The result of the Chi-square goodness-of-fit test for F_2 segregating frequency best conformed to the 15:1 ratio with a x^2 value of 4.79 ($x^2.050 - x^2.025$), indicating the involvement of a duplicate dominant epistatic as the most likey model for resistance to *P. capsici* in *S. habrochaites* 'PI 127827'. The slight excess of susceptible plants might be due to the harsh inoculation conditions plus variable levels of penetrance as indicate by few symptomatic plants also observed in the F_1 generation (**Table 1**). We tentatively named these two loci as *Phythphthora capsici resistance 1* (*Phcr*1) and *Phythphthora capsici resistance 2* (*Phcr*2). Under this duplicate epistatic model, the resistant phenotype, to be expressed, needs only one allele of the each individual dominant (and independent) gene in heterozygous condition (either *Phcr*1_ or *Phcr*2_). Susceptibility will be fully expressed in the double recessive phenotype *phcr*1*phcr*1/*phcr*2 *phcr*2.

Discusssion

The development of elite lines with broad-spectrum resistance to *P. capsici* isolates has been difficult as indicated by the lack of commercial cultivars with suitable levels of resistance in virtually all host crops of this pathogen (Lamour *et al.* 2012b; Quesada-Ocampo *et al.* 2016; Saltos *et al*. 2022). This is because *P. capsici* harbors enormous genetic diversity and wide host range, which is a strong biological indicator of a pathogen genetically difficult to control (Lamour *et al*. 2012a; Guerrero-Aguilar *et al.* 2022; Parada-Rojas *et al.* 2022). For this reason, the inheritance of a resistance source that is effective against a wide array of *P. capsici* isolates such *S. habrochaites* 'PI 127827' is a crucial piece of information, providing a more precise understanding of the genetic basis of this trait. This knowledge will facilitate the subsequent introgression into commercial tomato accessions.

In fact, the resistance to a wide array of *P. capsici* isolates is under control of different genetic mechanisms according to the accession of the host species and to the pathogen isolate in Cucurbitaceae hosts. In melon (*Cucumis melo* L.), resistance is simple, being controlled by a

dominant gene (Wang *et al.* 2020), whereas in *Cucurbita* species there is a report indicating that three dominant genes are necessary to confer resistance (Padley *et al.* 2009).

In *Capsicum* species (Solanaceae), a wide array of genetic mechanisms is underlying resistance to the pathogen involving single genes up to complex inheritance according to the interaction of the source of resistance and the *P. capsici* isolate (for review see Barchenger *et al*. 2018; Siddique *et al.* 2019; Sanogo *et al*. 2022). In addition, the presence of different physiological races/pathotypes of *P. capsici* and the employement isolates with low virulence could lead to misleading genetic analyses of the resistance mechanisms (Siddique *et al.* 2019). Bartual *et al*. (1991; 1994) observed significant effects of epistasis in diallel crosses involving seven partially resistant C. *annuum* accessions inoculated with different *P. capsici* isolates. It was observed in a distinct study that the resistance trait in *C. annuum* 'Criollo de Morellos 334' was under control of two major genes with dominant and recessive (13:3) epistasis (Reifschneider *et al.* 1992). Bnejdi *et al.* (2009) also found the involvement of epistasis in the resistant reaction of *C. annuum* 'Criollo de Morellos 334' in response to another *Phytophthora* species (*P. nicotianae*) and they observed that plant reaction is dependent upon the aggressiveness of the isolate. The greater the aggressiveness of the *P. nicotianae* isolate, the greater the effect of dominant epistasis in *C. annuum* 'Criollo de Morellos 334'. For the less aggressive isolates, the epistasis of resistance was not detected (Bnejdi *et al.* 2010). Individual recombinant inbred lines (RILs) derived from the cross 'Criollo de Morelos 334' *x* 'Early Jalapeno' were hybridized to each other to obtain F_1 and F_2 populations. When the F_2 populations were inoculated with a single race, ratios of 3:1 (resistant:susceptible) were obtained, indicating a single gene. When the F_2 populations were inoculated with a combination of two races, segregation ratios of 15:1 (resistant:susceptible) were observed in some populations with the presence of linkage between some of the resistance genes (Monroy-Barbosa and Bosland, 2008). In India, a breeding line was identified with resistance controlled by a major dominant gene along with a few minor genes (Kumar *et al.* 2021).

Different quantatitive trait loci (QTLs) in different genomic regions with the presence of epistasis influencing the resistance responses to *P. capsici* were also detected (Lefebvre and Palloix, 1996; Thabuis *et al.* 2004). Bonnet *et al.* (2007) identified polygenic resistance controlled by 12 Quantitative trait loci QTLs) with additive effects in *C. annuum* accessions. Siddique *et al.* (2019) detected three major-efect loci conferring broad-spectrum resistance to three isolates of *P. capsici*. These loci were located in the pepper chromosome P5 employing

traditional QTL mapping with genome-wide association studies (GWAS). In addition, QTLs with epistatic interactions and isolate-specific and environment-specific minor efects were detected on other chromosomes. Interestingly, clusters of candidate nucleotide binding site-leucine-rich repeat (NBS-LRR) and receptor-like kinase (RLK) genes were predicted within the QTL and GWAS regions encompassing *P. capsici* resistance factors. In *Solanum* (section *Lycopersicon*) germplasm, Quesada-Ocampo *et al.* (2016) characterized a QTL-like resistance to *P. capsici* in RILs derived from *S. habrochaites* 'LA 407', but they were not able to identify how many genes/genomic regions are involved.

Study conducted by Petry *et al.* (2017) was the first indication of the presence of races/pathotypes in *P. capsici Solanum* (section *Lycopersicon*) germplasm. In previous studies *S. habrochaites* 'PI 127827' and it displayed superior levels of immune-like resistance to a wide array of isolates from different regions of Brazil in comparison with *S. habrochaites* 'LA407' (Gonçalves-Pereira *et al.* 2023a; Gonçalves-Pereira *et al*. 2023b). Here, the chi-square goodness-of-fit test for F_2 segregating ratios using crosses with *S. habrochaites* 'PI 127827' indicated this ratios in accordance to a duplicate dominant epistatic (ratios of 15:1 resistance:susceptible).

Under this model, the resistant phenotype, to be fully expressed, needs only one of the two dominant genes in heterozygous condition. We tentatively named these two loci as *Phcr*1 and *Phcr*2. In *Capsicum*, duplicate dominant genes may control resistance under distinct inoculum pressures (Lee *et al*. 2012) and breeding lines carrying a combination of distinct and independent major genes or QTLs (Palma-Martínez *et al*. 2017). Duplicate dominant epistasis has been also detected in distinct pathosystems involving *Phytophthora* species. The resistance against *P. sojae* race 1 in the large-spectrum resistant soybean landrace 'PI 567139B' dispayed a good fit to 15:1 ratio, indicating the action of two independent dominant resistance genes, which were found to be located in genomic regions riches in NBS-LRR genes (Lin *et al.* 2013).

The smaller the number of genes, the more efficient is the breeding process for the effective transfer of resistance genes. This is true even when duplicated dominant epistasis is present (Viana, 2000). Therfore, from the tomato breeding standpoint, this relatively simple genetic control will facilitate the introgression into elite cultivated tomato lines of the superior levels of immune-like resistance displayed by *S. habrochaites* 'PI 127827' against a wide array of *P.*

capsici isolates (Gonçalves-Pereira *et al.* 2023a; Gonçalves-Pereira *et al*. 2023b). Obviously, the high expression of the the large-spectrum resistance phenotype of *S. habrochaites* 'PI 127827' make this accession also a useful source for the development of rootstocks (Reis *et al.* 2021). The confirmation of a relatively simple genetic control of the resistance of 'PI 127827' against the *P. capsici* 'PCp-182' is an outstanding towards the development of phenotypically stable and large-spectrum resistant cultivars. It is important to emphasize that the isolate *P. capsici* 'PCp-182' was found to be the isolate with the broadest virulence profile among the ones collected across many vegetable-producing regions of Brazil (Gonçalves-Pereira *et al*. 2023a; Gonçalves-Pereira *et al.* 2023b).

The use of molecular markers, in addition to the phenotypic assays, can increase the precision and efficiency of subsequent selection steps in plant breeding programs. Therefore, further studies aiming for the identification of molecular markers associated with resistance to *P. capsici* in *S. habrochaites* 'PI 127827' will facilitate breeding efforts. In fact, molecular analyses of the segregating progeny developed in this study is currently underway with the goal of identifying markers linked to the two alleles that confer resistance to *P. capsici*. The development of markers linked to *Phcr*1 and *Phcr*2 can be facilitated by the large number of *Solanum* (section *Lycopersicon*) genomes currently available (Gao *et al.* 2019). PCR-based codominant markers tightly linked to these loci would allow the distinction between individuals who are homozygous or heterozygous at each of the two loci required for resistance (Campos *et al*. 2021). The molecular analysis of the resistance loci characterized here may also explain the genomic structure and potential contribution of distinct genomic regions in the expression of this resistance. Epistasis requires looking for interaction effects among all genomic loci and highlights the influence of a genetic background in the expression of a given resistance trait (Fethi *et al.* 2011). In addition, the presence of epistatic interactions may imply the enhancement or attenuation of resistance gene expression and may also be affected over the course of crosses during the breeding process (Kim *et al.* 2008; Phillips, 2008).

In the present work, for the first time, a study on the genetic inheritance of a *Solanum* (section *Lycopersicon*) accession to *P. capsici* was analyzed under Brazilian conditions. Segregating patterns of populations derived from the resistance source *S. habrochaites* 'PI 127827' fitted to the 15:1 ratio, indicating the involvement of a duplicate dominant epistatic model for resistance against one *P. capsici* isolate. Under this model, the resistant phenotype expression

needs only one of the two dominant genes in heterozygous condition. We tentatively named these two loci as *Phythphthora capsici resistance* 1 (*Phcr*1) and 2 (*Phcr*2). Futher mapping studies will provide information about the classes of genes and mechanism involved in this resistance, if they represent, for instance, candidate nucleotide binding site-leucine-rich repeat (NBS-LRR) and receptor-like kinase (RLK) genes. Nevertheless, this relatively simple genetic control will facilitate the introgression of this trait into elite tomato lines.

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Table 1 – Segregation for resistance (R) and susceptibility (S) to *Phytophthora capsici* in populations derived from two contrasting *Solanum* (section *Lycopersicon*) accessions: the highly susceptible female parent *S. lycopersicum* cultivar 'Ponderosa' and the highly resistant male parent (= pollen donor) *S. habrochaites* 'PI 127827'. Reciprocal crosses were not done due to unilateral incompatibility when using *S. habrochaites* as female parent. The contrasting parental lines and their corresponding F_1 and F_2 populations were inoculated with the *P. capsici* isolate PCp-182.

Generation	**Observed**			**Expected**		**Hypotheses**	x^2	**P (%)**
	R	**S**	**Total**	**R**	**S**			
PI 127827	25	0	25	25	0	---	---	---
Ponderosa	0	25	25	0	25	---	---	---
F1	68	2	70	70	0	---	---	---
F2	**226**	**24**	250	**234**	**16**	**15:1**	**4.79**	**2.87**
F2	226	24	250	188	63	3:1	31.62	0.00
F2	226	24	250	141	109	9:7	118.47	0.00
F2	226	24	250	203	47	13:3	13.74	0.02
F2	226	24	250	145	105	37:27	108.85	0.00
F2	226	24	250	246	4	63:1	105.00	0.00

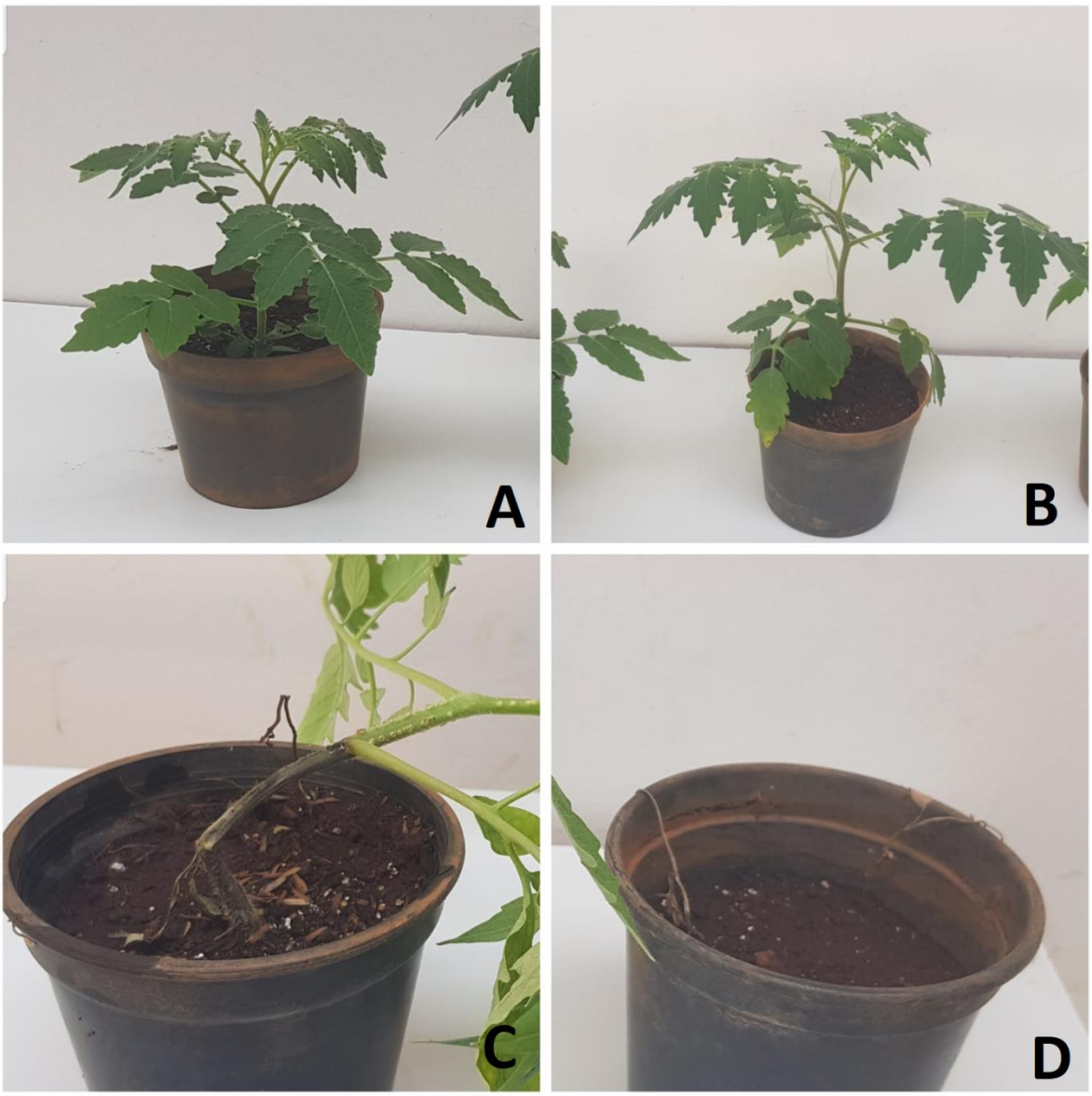

Figure 1 – Response of accession *Solanum habrochaites* 'PI 127827' and *S. lycopersicum* 'Ponderosa' after inoculation with *Phytophthora capsici* ('PCp-182' isolate). Plants of *S. habrochaites* 'PI 127827' **(A)** and the majority of the plants of the F_1 generation **(B)** were free of symptoms after PCp-182 inoculation. The cultivar 'Ponderosa' showed severe symptoms of girdling in the crown region that rapidly expands to the stem **(C)** followed by complete plant collapse **(D).**

CONCLUSÕES GERAIS

- A partir desse estudo foi possível catalogar parcialmente a diversidade genética de isolados de *P. capsici* de Solanaceae e Cucurbitaceae quanto aos grupos de compatibilidade, haplótipos, capacidade de causar doença em frutos e plântulas de tomateiro e pimentão.
- A introgressão/incorporação dos fatores de resistência dos acessos 'Hawaii-7996' e *S. habrochaites* em linhagens de elite seria uma estratégia de melhoramento promissora para o desenvolvimento de cultivares/híbridos/porta-enxertos superiores de tomateiro para condições Neotropicais.
- *S. lycopersicum* 'Hawaii-7996' e *S. habrochaites* 'PI-127826' e 'PI-127827' apresentaram resistência contra uma ampla gama de isolados.
- Oito patótipos foram identificados em uma amostra de dez isolados de acordo com seus padrões únicos de interação com nove acessos hospedeiros.
- O controle genético do cruzamento entre o acesso resistente 'PI-127827' e o cultivar comercial suscetível 'Ponderosa' permitiu encontrar um modelo epistático dominate duplicado para resistência, com proporção 15:1 na população F_2. Esse controle genético simplificado vai favorecer a incorporação desta característica em linhagens elite de tomateiro.