Received: 26 April 2023 | Accepted: 21 September 2023

DOI: 10.1111/ppa.13803

Plant Pathology WILEY

REVIEW ARTICLE

Current understanding of the *Diaporthe/Phomopsis* complex causing soybean stem canker: A focus on molecular aspects of the interaction

Eilyn Mena[1] | Silvina Stewart[2] | Marcos Montesano[1,3] | Inés Ponce de León[1]

[1]Departamento de Biología Molecular, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

[2]Sección Protección Vegetal, Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay

[3]Laboratorio de Fisiología Vegetal, Centro de Investigaciones Nucleares, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

Correspondence
Inés Ponce de León, Departamento de Biología Molecular, Instituto de Investigaciones Biológicas Clemente Estable, Avenida Italia 3318, Montevideo, 11600, Uruguay.
Email: iponce@iibce.edu.uy

Funding information
Programa de Desarrollo de las Ciencias Básicas (PEDECIBA); Programa para Grupo de I + D Comisión Sectorial de Investigación Científica, Universidad de la República

Abstract

Soybean stem canker (SSC) is an important disease caused by different *Diaporthe* spp., including *D. aspalathi*, *D. caulivora* and *D. longicolla*, that leads to soybean (*Glycine max*) yield losses around the world. Most studies have been focused on the morphological characterization and molecular identification of *Diaporthe* spp. present in SSC lesions. Several soybean resistance loci to *Diaporthe* spp. causing SSC have been identified, although the molecular identities of the resistance genes are at present unknown. In this review, we summarize the current knowledge on SSC disease, the molecular characterization of *Diaporthe* spp. and their evolutionary relationships. We highlight how recent genomic and transcriptomic information is allowing significant progress in our understanding of the molecular components and mechanisms underlying *Diaporthe* infection strategies as well as soybean disease resistance. The information generated, combined with available resources enabling functional genomics, will contribute to the development of breeding strategies for disease resistance, leading to a more sustainable agriculture.

KEYWORDS
disease symptoms, genomes, pathogenesis, plant disease, resistance genes, soybean stem canker

1 | INTRODUCTION

Diaporthe/Phomopsis complex includes agriculturally important plant pathogens, endophytes and saprophytes with a wide range of plant hosts in tropical and temperate regions (Cao et al., 2022). The most studied *Diaporthe* spp. are those associated with soybean (Santos et al., 2011; Zhang et al., 1998), sunflower (Thompson et al., 2011), citrus (Udayanga et al., 2014), grapes (Baumgartner et al., 2013; van Niekerk et al., 2005), sweet potato (Huang et al., 2021) and blueberry (Hilário et al., 2021).

Soybean (*Glycine max*) is cultivated in more than 100 countries around the world, principally in Brazil, the United States, Argentina and India (FAOSTAT, 2023). Fungal *Diaporthe* spp. cause significant yield losses in soybean worldwide, leading to pod and stem blight, seed decay and stem canker (Backman et al., 1985; Fernández et al., 1999). *Diaporthe sojae* is the causal agent of pod and stem blight, while *D. longicolla* (syn. *Phomopsis longicolla*) is the primary agent of Phomopsis seed decay (PSD; Fernández & Hanlin, 1996; Sinclair, 1999). Soybean stem canker (SSC) is caused by *D. aspalathi* (syn. *D. phaseolorum* var. *meridionalis*) and *D. caulivora* (syn. *D. phaseolorum* var. *caulivora*) (Fernández et al., 1999; Pioli et al., 2003). Recently, *D. longicolla* has been associated with stem canker lesions, and inoculation assays performed under field and laboratory conditions confirmed that this species causes SSC (Gebreil et al., 2015; Ghimire et al., 2019; Mathew, Castlebury, et al., 2015; Mena et al., 2020; Olson et al., 2015). Field losses due to SSC can reach levels of 80%–100% (Backman et al., 1985; Krausz & Fortnum, 1983). Therefore, susceptible cultivars have been replaced with less susceptible or resistant cultivars carrying loci that significantly lower SSC incidence (Lin et al., 2022; Ploetz

& Shokes, 1985). Several loci responsible for resistance to *D. aspalathi* and *D. longicolla* have been identified and incorporated in breeding programmes (Lin et al., 2022; Maldonado dos Santos et al., 2019). Nevertheless, selection pressure given by the incorporation of soybean resistance loci to *D. aspalathi* has promoted the expansion of SSC caused by *D. caulivora* in different countries (Abdelmagid et al., 2019; Pioli et al., 2002).

Most studies on SSC have focused on morphological characteristics and molecular identification of *Diaporthe* spp. present in canker lesions of infected plants in different countries (Ghimire et al., 2019; Mathew, Castlebury, et al., 2015; Mena et al., 2020). However, less information is available on the molecular mechanisms involved in soybean–*Diaporthe* interactions. This review provides an overview of the recent findings in *Diaporthe* species causing SSC and highlights how recent genomic- and transcriptomic-based research has allowed the identification of putative pathogenicity genes and plant defence strategies leading to plant resistance. This information could stimulate the development of novel diagnostics and control methods, leading to improvements of resistance-breeding programmes.

2 | THE PATHOGENS

2.1 | History of distribution

SSC was first reported in Iowa, United States, in the 1940s, where the disease was caused by *D. phaseolorum* var. *batatatis* (Crall, 1950; Welch & Gilman, 1948). *D. phaseolorum* var. *batatatis* was renamed as *D. phaseolorum* var. *caulivora* (Athow & Caldwell, 1954). After this first report, SSC was detected in the 1970s in the southern states of the United States (Mississippi and Alabama) (Backman et al., 1981; Keeling, 1982), and by 1984, the disease had spread to all southern soybean-producing areas (Snow et al., 1984), where it became an emerging disease problem in the United States (Backman et al., 1985). The name *D. phaseolorum* f. sp. *meridionalis* was proposed for the causal organism of stem canker in the South (Morgan-Jones, 1989), and later, it was renamed to *D. aspalathi* (van Rensburg et al., 2006). Pathogenicity tests allow distinction among isolates with different aggressiveness, and temperature preferences differentiate between *D. aspalathi* causing stem canker in the southern United States and *D. caulivora* causing stem canker in the northern United States (Keeling, 1982, 1985, 1988; Morgan-Jones, 1989). *D. caulivora* prefers cooler temperatures of 20–25°C, while *D. aspalathi* and *D. longicolla* prefer warmer temperatures of 25–30°C (Keeling, 1988; Mengistu et al., 2009).

Isolates of *Diaporthe/Phomopsis* species causing SSC have also been found in other countries, including Canada (Abdelmagid et al., 2019; Hildebrand, 1956), Australia (Stovold & Francis, 1987), Ghana (Asante et al., 1998), China (Chen et al., 2020; Cui et al., 2009; Zhao et al., 2022), Korea (Oh, 1998; Sun et al., 2012), Croatia (Santos et al., 2011; Vratarić et al., 1998), and France, Italy and Spain (Bertolini & Tanzi, 1987; EPPO, 2021; Hilário et al., 2021; Hissek et al., 2017).

In South America, the main soybean-producing countries are Brazil, Argentina, Paraguay, Bolivia and Uruguay. Last year soybean production reached 196,866,713 tonnes in the region (FAOSTAT, 2023). The first reports of SSC caused by *D. aspalathi* in South America were in 1989, 1992, 1994 and 1997 in Brazil, Paraguay, Bolivia and Argentina, respectively (Costamilan et al., 2008; Jaccoud-Filho et al., 1997; Pioli et al., 2001; Sato et al., 1993). The presence of *D. caulivora* in soybean fields was described for the first time in Argentina in 1999, in Brazil in 2006 and in Uruguay in 2015 (Costamilan et al., 2008; Pioli et al., 2001; Stewart, 2015). The disease caused by *D. caulivora* spread throughout the productive area of Argentina, where it coexists with *D. aspalathi* (Pioli et al., 2002). Stem canker expansion produced yield losses close to 100% in some fields of Brazil and Argentina (Grijalba & Ridao, 2012; Yorinori, 1996). Recently, SSC, mainly caused by *D. caulivora*, has been one of the most destructive soybean diseases in South America, with a prevalence of 50%, 61% and 83% in Paraguay, Argentina and Uruguay, respectively (Sánchez et al., 2015; Stewart, 2015; Wrather et al., 1997). The presence of *D. caulivora* isolates in a high proportion of SSC lesions is probably due to the use of resistant soybean genotypes carrying resistance genes effective for *D. aspalathi* but not for *D. caulivora* (Peruzzo et al., 2019; Pioli et al., 2003; Stewart, 2015). A third *Diaporthe* species recovered at high frequencies from SSC lesions in different countries was *D. longicolla* (Harrington et al., 2000; Lu et al., 2010; Sinclair, 1999; Xue et al., 2007). *D. longicolla* isolates were able to produce infection and SSC lesions in susceptible soybean plants (Ghimire et al., 2019; Mena et al., 2020). In addition to *D. caulivora* and *D. longicolla*, isolates of *D. miriciae* (syn. *D. ueckerae*) were obtained from symptomatic soybean plants with stem canker in Uruguay, each representing 42%, 37% and 15%, respectively, of the total *Diaporthe* isolates (Mena et al., 2020). *D. gulyae* and *D. miriciae* were also detected in SSC symptomatic soybean plants in North Dakota, United States, and in Meta, Colombia (López-Cardona et al., 2021; Mathew et al., 2018). Recently, *D. cucurbitae* was recovered from symptomatic canker lesions of soybean plants in Minnesota, United States, and for the first time it was confirmed to be an SSC-causing pathogen (Floyd & Malvick, 2022). These findings indicate that other *Diaporthe* spp., in addition to *D. caulivora*, *D. aspalathi* and *D. longicolla*, infect soybean and produce SSC symptoms.

2.2 | Taxonomy and molecular characterization of *Diaporthe* species

Diaporthe spp. belong to the phylum Ascomycota of the fungal kingdom, class *Sordariomycetes*, subclass *Sordariomycetidae*, order *Diaporthales*, family *Diaporthaceae*. *Diaporthe* genus was established as *Diaporthaceae* by Fuckel (1867) and Nitschke (1870), and *Phomopsis* (Sacc.) Bubák (1905) was introduced as the asexual morph (Species Fungorum Plus [https://www.gbif.org/es/species/2566075]). The family *Diaporthaceae* was established by von

Höhnel (1917) and was accommodated in the order *Diaporthales*, which includes 12 families with about 50 genera (Rossman et al., 2015).

The *Diaphorthe*/*Phomopsis* complex includes hemibiotrophic fungi with two phases: the imperfect, asexual or anamorphic *P. phaseoli*, and the perfect, sexual or teleomorphic *D. phaseolorum* (Santos et al., 2011). Currently, more than 1100 epithets for *Diaporthe* and 900 for *Phomopsis* are listed in the Index Fungorum database (http://www.indexfungorum.org/), with names often based on host association. In addition, 1260 and 1050 *Diaporthe* and *Phomopsis* spp., respectively, are listed in the Mycobank database (http://www.mycobank.org/). However, some of these isolates represent the same or synonymous species with a sexual and an asexual morph. In order to assign only one name for each fungus, *Diaporthe* is recommended over *Phomopsis* (Rossman et al., 2015; Xu et al., 2021).

Growth characteristics of *D. aspalathi*, *D. caulivora* and *D. longicolla* mycelia in culture media are similar and some differences in morphological characteristics of their reproductive structures (perithecia and ascospores) have been observed (Figure 1). On potato dextrose agar (PDA), colonies of the three pathogens are white with occasional yellow-brown areas (Figure 1a). On the reverse side of PDA plates, the colonies have different pigmentation in striate zones. The *Diaporthe* genus is characterized by immersed ascomata with perithecia that appear singly or clustered in groups (Gomes et al., 2013; Mena et al., 2020). On PDA, *D. aspalathi* perithecia have dark globose bases, while *D. longicolla* perithecia are black with three or more protruding beaks, and *D. caulivora* has perithecia with thin beaks that are dispersed singly or in groups of two or three (Figure 1b). Ascospores of the three *Diaporthe* spp. are translucent, ellipsoidal to fusoid, medianly septated and biguttulate (Gomes et al., 2013; Mena et al., 2020; Udayanga et al., 2011) (Figure 1c). Ascospores may have different lengths and widths within and between species, and differences according to the regions of collection also exist (Brumer et al., 2018). Some authors refer to the production of conidiomata and absence of pycnidia in *D. caulivora* grown on PDA plates (Athow & Caldwell, 1954; Pioli et al., 2003; Santos et al., 2011). However, the presence of alpha-conidia in soybean tissues or in culture medium containing soybean stems has been observed for some *D. caulivora* isolates (Brumer et al., 2018; Fernández et al., 1999; Mena et al., 2020; Sun et al., 2012). Moreover, *D. longicolla* isolates differ from *D. aspalathi* and *D. caulivora* in having larger pycnidia, and longer conidiophores with alpha- and beta-conidia (Olson et al., 2015; Santos et al., 2011; Vidić et al., 2013). Some isolates of *D. longicolla* only produce alpha-conidia, while beta-conidia are absent (Hosseini et al., 2020). Alpha- and beta-conidia of *Diaporthe* spp. can be distinguished by their form and size; alpha-conidia are one-celled, biguttulate, ellipsoidal to oval, subcylindrical, fusiform, hyaline and pointed at both ends, while beta-conidia are single-celled, aseptate, filiform, curved at one end, eguttate and hyaline (Hosseini et al., 2020; Nishmitha et al., 2022). In *D. caulivora* and *D. longicolla*, sizes range from 1.3 to 4.0×3.7 to 8.5 μm for alpha-conidia and from 1.3 to 1.9×9.3 to 31.6 μm for beta-conidia (Hosseini et al., 2020; Nishmitha et al., 2022; Rupe, 2015).

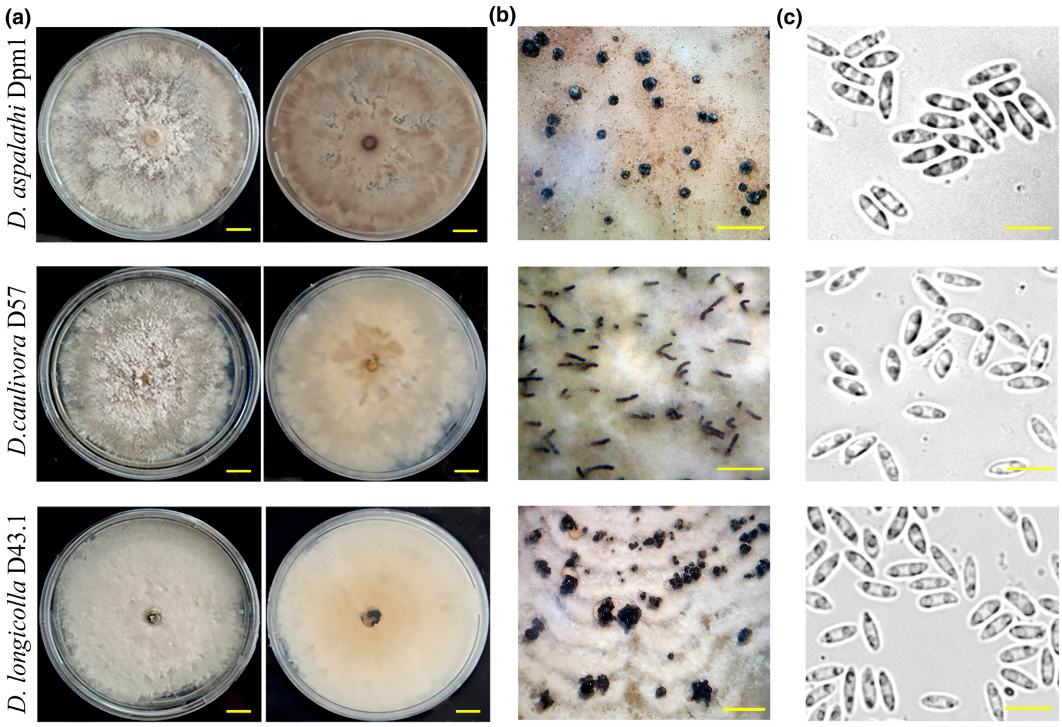

FIGURE 1 Morphology of *Diaporthe* pathogens causing soybean stem canker. (a) Cultures of *Diaporthe aspalathi* (isolate Dpm1), *D. caulivora* (isolate D57) and *D. longicolla* (isolate D43.1) showing different morphological characteristics grown on potato dextrose agar (PDA) for 7 days. Scale bars: 10 mm. (b) Perithecia structures of *D. aspalathi*, *D. caulivora* and *D. longicolla* on PDA. Scale bars: 10 mm. (c) Ascospores of *D. aspalathi*, *D. caulivora* and *D. longicolla*. Scale bars: 10 μm.

The use of morphological and developmental criteria or symptom development for *Diaporthe* taxonomy is not recommended due to variability among morphological traits (Gomes et al., 2013; Pioli et al., 2003; Zhang et al., 1998). Furthermore, SSC symptoms caused by the different *Diaporthe* spp. can be easily confused (Lu et al., 2010). Therefore, during the last few years, molecular markers have been developed to distinguish between *Diaporthe* spp. PCR-restriction fragment length polymorphisms (PCR-RFLP) allow differentiation between *D. aspalathi*, *D. caulivora* and *D. longicolla* according to the number and size of DNA fragments, using a combination of restriction enzymes after rDNA internal transcribed spacer (ITS) amplification (Brumer et al., 2018; Moleleki et al., 2002; Stewart, 2015). Likewise, the use of molecular analysis supported with DNA sequencing data, including multilocus phylogeny with rDNA ITS region, elongation factor 1-α (EF1-α), β-tubulin, actin and calmodulin genes, results in a reliable method to distinguish between closely related *Diaporthe* isolates (Mathew, Alananbeh, et al., 2015; Mena et al., 2020; Santos et al., 2011; Udayanga et al., 2014).

2.3 | Comparative genomics of *Diaporthe* species

At present, 31 *Diaporthe* spp. genomes have been sequenced, annotated and deposited in NCBI (https://www.ncbi.nlm.nih.gov/genome/?term=diaporthe), including five genomes of *Diaporthe* spp. capable of causing SSC. The first two genome sequences of *D. longicolla* were published in 2015 for isolates MSPL 10–6 and TWH P74, both obtained from soybean seeds in the United States (Li, Darwish, et al., 2015; Li, Song, et al., 2015; Li et al., 2017). These genome drafts were generated using Illumina short sequencing reads technologies and assembled into 985 scaffolds with a total size of 64.7 Mb for isolate TWH P74 (Li, Song, et al., 2015) and into 108 scaffolds and a total size of 66.7 Mb for isolate MSPL10-6 (Li, Darwish, et al., 2015; Li et al., 2017). The third *D. longicolla* genome sequence was published in 2021 for isolate YC2-1, which was obtained from a soybean stem with stem blight symptoms (Zhao et al., 2021). This genome sequence was obtained using Illumina and PacBio long sequencing technology that allows a more accurate assembly of genomic sequences. The YC2-1 genome has an estimated size of 63.1 Mb assembled into 87 scaffolds. Currently, only one genome of *D. aspalathi* has been sequenced (isolate MS-SSC91), which was obtained from soybean stems in the United States and sequenced using Illumina (Li et al., 2016). The MS-SSC91 genome assembly size was estimated as 55.0 Mb assembled into 1871 scaffolds. *D. caulivora* genomes were published more recently, in 2022 and 2023. *D. caulivora* isolate D57 was obtained from soybean stems with SSC symptoms in Uruguay fields, and the genome was sequenced with Pacbio, assembled into 10 scaffolds with a total size of 57.8 Mb (Mena et al., 2022). The second *D. caulivora* genome sequence was from the Russian Far Eastern isolate VNIIKR SE Dcaul3, obtained from soybean stems with SCC symptoms, sequenced with Illumina and assembled into 84 scaffolds with a genome size of 55.8 Mb (Muterko et al., 2023).

Comparison of the currently available genome data of these SSC-causing pathogens and other annotated *Diaporthe* genomes allows the elucidation of their biological and evolutionary relationships. Genomic evolution between *Diaporthe* spp. was evaluated by synteny that defines a common order of homologous genes in chromosome regions of different genomes. High syntenic similarity was observed in *D. caulivora* D57, *D. capsici*, *D. citri*, *D. destruens* and *D. phragmitis* genomes, indicating their close genetic relationship as shown by the conserved organization of their genomes (Mena et al., 2022). When *D. caulivora* VNIIKR SE Dcaul3 genome was compared with 14 other plant-pathogenic *Diaporthe* spp. genomes, the highest similarity in large-scale genome organization was with *D. aspalathi* (Muterko et al., 2023). However, the evolution of the *Diaporthe* genus is likely to be host-independent because both *D. caulivora* and *D. longicolla* infect soybean but exhibit long phylogenetic and genomic distance and differ significantly in genome organization, including distribution of interspersed repeats (transposable genomic elements), and GC content (Muterko et al., 2023). Consistently, a multigene phylogeny for these *Diaporthe* spp., based on 20,000 polymorphic sites of more than 100 orthologous genes, has indicated that *D. caulivora* is closely related to *D. aspalathi*, while *D. longicolla* groups with other *Diaporthe* spp. that infect other hosts (Muterko et al., 2023).

The number of predicted genes is 15,738, 16,279 and 16,597 in *D. longicolla*, 14,962 in *D. aspalathi*, and 15,666 and 18,385 in *D. caulivora* (Li, Darwish, et al., 2015; Li, Song, et al., 2015; Li et al., 2016; Mena et al., 2022; Muterko et al., 2023; Zhao et al., 2021). Differences in the number of predicted genes for different isolates of a single species could be due to the bioinformatics algorithms used. When applying the same algorithm and parameters in the Augustus web server, the number of predicted genes for *D. caulivora* isolate D57 and VNIIKR SE Dcaul3 had similar results (Muterko et al., 2023). In *D. longicolla* MSPL10-6 and *D. caulivora* D57, 9.64% and 9.72% of the predicted genes did not significantly match any known genes (Li et al., 2017; Mena et al., 2022) and could be considered species-specific genes. Moreover, the 15 plant-pathogenic *Diaporthe* spp. studied by Muterko et al. (2023) shared 6348 proteins (core proteins), while 502 proteins were shared between *D. caulivora* and *D. aspalathi* exclusively, and only four were present in the three soybean pathogens (*D. caulivora*, *D. aspalathi* and *D. longicolla*). These findings suggest that host adaptation and colonization strategies probably depend on core proteins rather than on species-specific proteins. Genome sequencing of new *Diaporthe* pathogens, together with comparative genomics, will provide new insights into the evolution of common and species-specific virulence factors among *Diaporthe* spp.

2.4 | Molecular basis of pathogenicity

Similar to many other pathogenic fungi, *Diaporthe* spp. capable of causing SSC rely on plant cell wall-degrading enzymes (PCWDEs), as well as enzymes involved in toxin production and secondary metabolites to infect the host plant and produce disease (Mena

et al., 2022). A large number of fungal proteins involved in pathogenesis are predicted to be secreted, and probably play important roles in degrading plant cell walls, causing plant cell death and degrading or interfering with antifungal activities of plant metabolites (Mena et al., 2022). Across *D. longicolla* TWH P74, *D. caulivora* D57 and *D. caulivora* VNIIKR SE Dcaul3, the predicted number of secreted proteins ranges from 1501 to 1695 (Li et al., 2017; Mena et al., 2022; Muterko et al., 2023). Among the secreted proteins of these *Diaporthe* spp., 30%–50% were carbohydrate-active enzymes (CAZymes), including glycoside hydrolases, glycosyltransferases, polysaccharide lyases and carbohydrate esterases, demonstrating the importance of efficiently degrading polysaccharides from the plant cell walls (Li et al., 2017; Mena et al., 2022; Muterko et al., 2023). A similar number of CAZymes were present in other plant-pathogenic *Diaporthe* spp. (Mena et al., 2022). Other proteins involved in *D. caulivora* pathogenesis include necrosis- and ethylene-inducing proteins (NEP), oxidoreductases, proteases and proteins involved in detoxification and transport of toxic compounds (Mena et al., 2022). Moreover, the identification of interacting proteins in the *D. longicolla* interactome highlights pathogenicity networks that comprise PCWDEs, glycoside hydrolases, cysteine-rich secretory proteins, cytochrome P450 domain-containing proteins, MAP kinases and transcription factors, which play important functions during pathogenesis in other fungi (Li et al., 2018). Comparative genomics between *D. caulivora*, *D. longicolla*, *D. capsici*, *D. citri*, *D. destruens* and *D. phragmitis* revealed a core secretome of 439 proteins present in all of these *Diaporthe* spp., including virulence factors such as PCWDEs, proteases, peptidases, lipases and peroxidases (Mena et al., 2022). Most of these secreted proteins are also present in other fungi and only 46 proteins were *Diaporthe*-specific, most of which have unknown functions. Moreover, 27 *D. caulivora*-specific secreted proteins were identified that have no hit with any other sequenced organisms. This emphasizes the need to perform functional studies to determine the role of these proteins during pathogenesis and plant colonization.

Effectors are virulence factors that are secreted by fungal pathogens to manipulate host cells, facilitate infection and interfere with host immunity (Toruño et al., 2016). By analysing the genome of *D. caulivora* D57, 133 putative candidate effectors were identified, including polysaccharide lyases, glycoside hydrolases, a pathogenesis-related protein, a hypersensitive response-inducing protein, peptidases, carbohydrate esterase and several hypothetical proteins (Mena et al., 2022). *D. caulivora* shared a high proportion of these candidate effectors with *D. longicolla*, *D. capsici*, *D. citri*, *D. destruens* and *D. phragmitis*, and nine of them were core effectors present in all *Diaporthe* spp., including a pectate lyase, a polysaccharide lyase, a 1,4-β-D-glucan cellobiohydrolase, a xylanase, a CAP22 protein and four hypothetical proteins (Table 1). The presence of core effector protein orthologues in other plant pathogens suggests that they may be involved in conserved processes vital for infection and colonization, and future research will provide more comprehensive clues of their molecular function in virulence.

Transcriptional profiling of *D. caulivora* D57 during soybean infection revealed that a high proportion of the upregulated genes (106 genes; 43%) correspond to genes encoding the core secretome, and several of them are represented in the pathogen–host interaction (PHI) database, which is consistent with their potential roles in pathogenic strategies (Mena et al., 2022). These include CFEM (Common in Fungal Extracellular Membrane) domain-containing proteins, NEPs, metalloproteases and acetylxylan esterases, which orchestrate the early stages of pathogen colonization (Mena et al., 2022). Increased expression of genes encoding subtilisin-like proteinases, aspartic proteinases and several proteases during *D. caulivora*-infected soybean suggests their possible involvement in degradation of plant defence proteins. Interestingly, some of these genes are present in the *Diaporthe* core secretome (Mena et al., 2022), suggesting that fungal proteases are important pathogenicity factors in *Diaporthe* spp. Additionally, expression patterns of upregulated genes and gene ontology enrichment analysis revealed that host infection strategies of *D. caulivora* depends on plant cell wall degradation and modification, detoxification of compounds, transporter activities and toxin production (Mena et al., 2022). However, functional analysis of pathogenesis-related *Diaporthe* proteins are needed as currently only two mitogen-activated protein kinase (MAPK) have been experimentally validated in *D. longicolla*, where knockout mutants displayed reduced virulence (Zhang et al., 2023). Further efforts on genomic and transcriptomic studies of *Diaporthe* pathogens will provide insights into fungal infection mechanism and colonization processes of soybean stems.

Polyketides constitute the main structural type of secondary metabolites in the *Diaporthe/Phomopsis* complex (Xu et al., 2021). Some of these polyketides have antifungal or antibacterial activities (Niaz et al., 2020; Silva et al., 2006). *D. helianthi* produces the polyketidic phytotoxin phomozin that caused SSC symptoms on leaves, and polyketide synthase (PKS) mutants exhibit reduced virulence during sunflower infection (Ruocco et al., 2018). In addition, 11 genes encoding PKS involved in toxin production in other fungi were induced during soybean infection with *D. caulivora*, which is consistent with PKS involvement in pathogenicity (Mena et al., 2022). This finding is in accordance with the possible involvement of phytotoxin(s) produced by *D. caulivora* during SCC development (Lalitha et al., 1989).

3 | SOYBEAN STEM CANKER

3.1 | Disease cycle

Diaporthe spp. are considered monocyclic pathogens, and the stem canker disease cycle consists of five main steps: (1) production of perithecia and ascospores, (2) ascospore dissemination, (3) contact and penetration, (4) infection, and (5) colonization (Figure 2). The disease cycle begins with the production of reproductive structures such as perithecia or pycnidial conidiomata (Sun et al., 2012). Ascospores actively released from perithecia disperse by rain and wind (Backman et al., 1985). Once the spores make contact with

TABLE 1 Overview of some apoplastic effector proteins identified in *Diaporthe caulivora* and *D. longicolla* genomes, with functionally characterized homologous in the Pathogen–Host Interaction database (PHI-base) (Mena et al., 2022).

Diaporthe species	Effector candidates	Homologous gene name (PHI-base)
Core effectors in *D. caulivora*, *D. capsici*, *D. citri*, *D. destruens*, *D. longicolla* and *D. phragmitis*	Pectate lyase	PELA
	Polysaccharide lyase family 3 protein	PELA
	1,4-β-D-glucan cellobiohydrolase A	cel2
	Xylanase G1	XYN11A
	Protein CAP22	–
	Hypothetical protein UCDDA912_g05111	–
	Hypothetical protein P154DRAFT_381873	–
	Hypothetical protein DHEL01_v209281	–
	Hypothetical protein CFIO01_13625	–
D. caulivora and *D. longicolla*	Pectate lyase F	PELD
	Glycoside hydrolase	Plegl1
	Glycoside hydrolase family 11 protein	XYL3
	Putative sterigmatocystin biosynthesis peroxidase stcC	MoHPX1
	Putative proline-rich antigen	–
	Putative aldehyde dehydrogenase	–
	Cellulose binding CEL1	MoCDIP4
	Carbohydrate-binding module family 1 protein	PITG_16135
	Carbohydrate-binding module family 50 protein	Blys8
	Fungal hydrophobin	MHP1
	Intracellular hyphae protein 1	SPL_1
D. longicolla	Carbohydrate esterase family 5 protein	MfCUT1
	Starch-binding domain-containing protein	SGA1
	Acetylxylan esterase 2	PBC1
	Cell wall protein PhiA	BbCwp_(BBA_03493)
	Fungal cellulose binding domain-containing protein	MoCDIP4
D. caulivora	Chitin deacetylase	CDA
	Putative cytochrome p450	–
	Peptidase S41 family protein	–
	Hypothetical protein UCRNP2_6738	–
	Hypothetical protein CSIM01_13334	–
	Hypothetical protein F66182_6057	–
	HET-domain-containing protein	–
	Hypothetical protein DHEL01_v212810	–
	Uncharacterized protein INS49_012633	–

the plant tissue, penetration and infection occur through wounds or natural entry points such as stomata or trichomes of leaves, stems and soybean seeds (Backman et al., 1985; Campbell et al., 2017; Kmetz et al., 1978; Mena et al., 2020). During the first days, spores germinate under high humidity conditions or water films present on the plant tissue (Mengistu & Heatherly, 2006). Plant pathogens of the genus *Diaporthe* may or may not form appressoria for host penetration. For example, appressoria have been observed in pod and soybean seed coat infected with *D. longicolla* (Baker & Minor, 1987). However, *D. sojae* conidia attach to soybean tissues, germinate and penetrate via stomata with or without forming appressoria and not via the cuticle (Kulik, 1988). In contrast, *D. toxica* conidia germinate on the plant surface of narrow-leafed lupins forming short germ tubes, which enlarge at the apices to form appressoria and directly penetrate the host cuticle (Shankar et al., 1998). Although appressoria of *D. caulivora* were not detected during soybean stem colonization, induction of CAP22-encoding gene was observed (Mena et al., 2022), which is highly expressed during appressoria formation in other fungi (Hwang & Kolattukudy, 1995). Because CAP22 is a core effector (Mena et al., 2022), further studies will underpin the

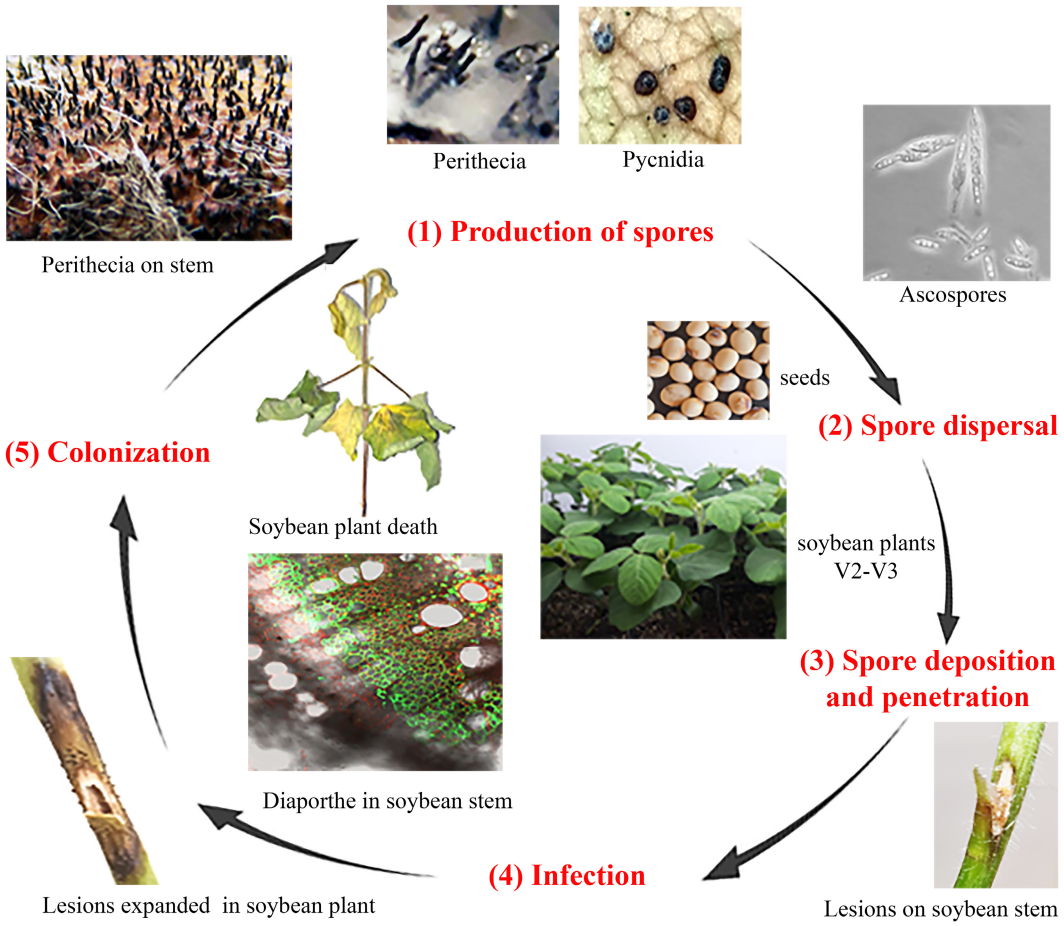

FIGURE 2 Representative life cycle of *Diaporthe* species causing soybean stem canker disease. Colonization of *Diaporthe caulivora* in a soybean stem section is visualized by staining with wheat germ agglutinin–Alexa Fluor 488 conjugate (WGA-AF488) (green: fungal cell wall) and PI (red: plant cell membrane/walls).

importance of this protein in appressoria development, host penetration and virulence.

Diaporthe spp. causing SSC colonize the vascular tissues, and under controlled conditions, *D. caulivora* has been demonstrated to colonize the cortex, phloem and xylem by 72 and 96 hours postinoculation (hpi) (Mena et al., 2020). At 7 days postinoculation (dpi), *D. caulivora* hyphae were able to progress through the vessels and spread via the collenchyma, pith and cortex along the stem (Mena et al., 2020). Phloem and xylem tissue colonization blocks the entire vascular system and degrades the stem pith. SSC lesions are covered with pycnidia that develop and remain on infected stem debris (Hilty, 1991). Although sporulation has been reported in tissues debris, the production of secondary inoculum from pycnidia during the season probably does not affect disease development or yield loss (Rupe, 2015). *Diaporthe* infections can also be the direct result of planting infected soybean seeds (Roth et al., 2020). Seed transmission rates are generally less than 1% for *D. aspalathi*, while these rates for *D. caulivora* can reach 10% to 20% (Rupe, 2015). Disease establishment depends fundamentally on soybean genetic susceptibility and the occurrence of conducive environmental conditions during the early stages of the infection process (Lu et al., 2010; Rupe, 2015).

3.2 | Symptoms

Soybean plants are infected by *Diaporthe* spp. at any developmental stage, although infection generally occurs during the early vegetative growth (Backman et al., 1985; Smith & Backman, 1988). Plants with symptoms of SSC can usually be identified in patches or randomly scattered within the soybean field (Figure 3a). Symptoms of infection are associated with reddish-brown discolouration and necrosis of the lower half of the stem (Figure 3b), and withered and dried brown leaves that remain attached to the plant. When the first symptoms appear, lesions consist of small points (1–2 mm) on the main stem at a lower node. During disease progression, lesions grow in both directions, and they become elongated and brown along the main stem or towards the lateral branches (Figure 3b). In general, canker lesions run along one side of the stem while the rest of the stem tissue remains green (Mena et al., 2020; Pioli et al., 2003). However, with a high inoculum source or in very susceptible plants, lesions coalesce, and the brown colouration appears on the entire stem with edges to the lesions no longer obvious. In addition, interveinal chlorosis and necrosis of the leaves are frequently visible (Lu et al., 2010; Mena et al., 2020; Pioli et al., 2003). Severe infections lead to necrosis of

FIGURE 3 Stem canker symptoms in soybean tissues. (a,b) Soybean stem canker (SSC) symptoms on soybean stem in the field. (c) Symptoms on soybean stems artificially inoculated with potato dextrose agar plugs containing mycelium of *Diaporthe aspalathi*, *D. caulivora* and *D. longicolla* at 7 days postinoculation under controlled conditions. Arrows in (a) indicate plants with SSC, and in (b) disease symptoms in the main stem and lateral branches. A white circle in (c) indicates *D. longicolla* perithecia growing on the infected stem.

the conductive vessels, which blocks the flow of water through the xylem leading to cell death in the upper portion of the stem and the death of the plant (Sánchez et al., 2015).

Under experimental conditions, some differences in the symptomatology exist depending on the causal agent and the inoculation method. When soybean stems are inoculated with *Diaporthe* mycelial plugs in wounded stems, stem canker lesions caused by *D. aspalathi* and *D. caulivora* present diffuse borders, while inoculations with *D. longicolla* exhibit lesions with more defined borders (Figure 3c). Infection by the three *Diaporthe* spp. produces brown dry lesions, which are darker with *D. caulivora* and *D. longicolla*, and perithecia are only visible after inoculation with *D. longicolla* (Figure 3c). Black pycnidia are also observed after stem inoculation with *D. longicolla* mycelial plugs (Mathew, Castlebury, et al., 2015). *D. caulivora*, *D. longicolla* and *D. aspalathi* produce elongated lesions and can lead to girdling of the stem when the toothpick and stem-wound inoculation methods are used (Ghimire et al., 2019; Mena et al., 2020). Moreover, *D. longicolla* inoculation of soybean plants by the toothpick method at vegetative stages V3–V4, when plants have the third and fourth unrolled and fully developed trifoliate leaves, respectively (Fehr et al., 1971), produces symptoms that include thin black lines on the lower stem that occasionally form irregular circles (Olson et al., 2015). Under field conditions, the association of a certain *Diaporthe* species to disease development can be challenging due to similarities in symptoms (Rupe, 2015). In addition, different *Diaporthe* spp. can usually coexist in the same lesion and cause disease by co-infection (Pioli et al., 2001; Santos et al., 2011). However, subtle differences in symptomatology can offer clues for *D. caulivora* and *D. aspalathi* differentiation. *D. caulivora* produces sunken, dark-brown canker lesions that appear on the lower nodes, which elongate and eventually girdle the stem, leading to plant wilting and death (Fernández et al., 1999). *D. aspalathi* causes canker lesions that are more delimited and rarely girdle the stem (Fernández et al., 1999). Similar foliar symptoms, such as interveinal chlorosis and necrosis, occur with both *Diaporthe* spp., and generally, leaves remain attached to the stem after plant death (Fernández et al., 1999). Furthermore, plant phenological stages influence *Diaporthe* colonization and symptom development. Inoculations of plants at V1 growth stage, with presence of the first unrolled and fully developed trifoliate leaf (Fehr

et al., 1971), resulted in significantly more snapped stems than older seedlings (Campbell et al., 2017). For this reason, inoculations of seedlings at growth stage V2 and V3 were more appropriate for consistent development of SSC and low amounts of stem snapping (Campbell et al., 2017). Moreover, disease incidence and severity with *D. aspalathi* is higher when infection occurs at V3 (Bowers & Russin, 1999; Smith & Backman, 1989). In the field, exposure to inoculum at V3 is associated with significant disease severity, which is progressively reduced at V4–V10 (plants with 4–10 unrolled and fully developed trifoliate leaves) (Smith & Backman, 1989).

Under controlled conditions and stem-wound inoculation, lesions started to appear at 3 dpi and plants died at 14 dpi (Mena et al., 2020). Inoculation of plants with ascospores or conidia in greenhouse experiments leads to slower development of symptoms, which were visible at reproductive stages (61–75 dpi) (Ploetz & Shokes, 1985). Similarly, under field conditions, disease development usually has high impact on plants during the reproductive stages (Fehr et al., 1971; Rupe, 2015), and the appearance of symptoms is affected by environmental conditions such as temperature, humidity or drought (Roth et al., 2020; Rupe, 2015). Soybean cultivars exhibit different levels of resistance depending on the isolate used during the inoculation process, providing evidence of pathogenic variability (Pioli et al., 2003). The identification of virulence profiles among circulating *Diaporthe* isolates is essential to understand the dynamic of the disease. In other well-studied pathosystems such as *Phytophthora sojae* and soybean, different countries conduct regular surveys to monitor changes in virulence towards resistance genes and pathotype diversity (Dorrance, 2018). In fact, *D. caulivora*, *D. aspalathi* and *D. longicolla* exhibit pathogenic variability on susceptible soybean plants and in soybean genotypes carrying different resistance loci to SSC (Brumer et al., 2018; Ghimire et al., 2019; Lu et al., 2010; Mena et al., 2020). Based on molecular and pathogenicity assays on soybean genotypes with resistance loci to *D. aspalathi*, four physiological races of *D. aspalathi* were identified in the core soybean-producing area of Argentina (Pioli et al., 2003). Similar results were obtained in Brazil where at least three races of *D. aspalathi* were detected (Brumer et al., 2018). Thus, selective pressure caused by the incorporation of plant resistance genes to SSC in soybean cultivars could be influencing the change in virulence among *Diaporthe* spp. in soybean fields.

3.3 | Host plant species

Diaporthe/*Phomopsis* spp. may use more than one plant species as a host (van Niekerk et al., 2005), either as endophytes, pathogens or saprobes (Santos et al., 2011; Thompson et al., 2011). In addition to soybean, *D. aspalathi* isolates have been obtained from other hosts, including blue lupin (Yorinori, 1990) and rooibos (van Rensburg et al., 2006). *D. caulivora* has also been found in cutleaf teasel (Santos et al., 2011), velvetleaf (Vrandecic et al., 2005), slender aster, prostrate knotweed (Mengistu et al., 2007) and sunflower (Brumer et al., 2018). Similarly, *D. longicolla* isolates have been found in several plants such as cowpea, giant ragweed, spotted spurge, curly dock and common cocklebur (Roy et al., 1997), as well as velvetleaf (Vrandecic et al., 2004), peanut (Sanogo & Etarock, 2009), eggplant (Shu et al., 2014) and dry edible beans and peas (Mathew, Castlebury, et al., 2015). In addition to the host plants from which the pathogens were isolated in nature, *Diaporthe* spp. are able to infect a range of other plants when artificially inoculated (Li et al., 2010). This is the case for *D. caulivora*, *D. longicolla*, *D. gulyae*, *D. miriciae*, *D. cucurbitae* and *D. helianthi*, which were able to cause stem canker in soybean and sunflower upon inoculation assays (Floyd & Malvick, 2022; López-Cardona et al., 2021; Mathew et al., 2018; Mena et al., 2020). Survival and perithecia development of *D. caulivora* on artificially infested stem pieces of other hosts, including maize, sorghum, sunflower, potato and wheat, suggests that other crops can serve as reservoirs for overwintering inoculum (Grijalba & Ridao, 2012).

4 | SOYBEAN STEM CANKER MANAGEMENT

Disease control is often carried out through cultural management such as early planting dates, disease-free seeds, crop rotation with nonhost crops, elimination of inoculum sources by practices such as tillage, and adjusting row width and population density (Freitas et al., 2002; Roth et al., 2020; Rothrock et al., 1985). These strategies contribute to reducing disease severity but their efficacy is limited (Fernández et al., 1999; Roth et al., 2020). Chemical fungicides used as seed treatments and/or foliar applications are adopted practices for controlling SSC, and include benomil, carboxin, carbendazin, thiabendazol, strobilurin, pyraclostrobin and tebuconazol (Floyd & Malvick, 2022; Yorinori, 1996). However, efficacy is limited when used alone, and for some products, like Edamame, chemical applications are not recommended in food-grade crops used for human consumption (Benavidez et al., 2010). Other sustainable alternatives are the use of short-maturity soybean varieties and SSC-resistant cultivars (Ivancovich & Botta, 2003). In fact, SSC is effectively controlled by the introgression of resistance loci in elite cultivars. For example, resistance loci to *D. aspalathi* are present in most of the Brazilian cultivars released after 1996 (Maldonado dos Santos et al., 2022).

4.1 | Host resistance

Plants have innate immune defences to counteract pathogen attacks, known as pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). Both PTI and ETI activate a number of overlapping defence reactions such as mitogen-activated protein kinase (MAPK) cascades, calcium influx, reactive oxygen species (ROS) accumulation, callose deposition, hormone synthesis and transcriptional reprogramming (Ngou et al., 2022). Plasma membrane pattern-recognition receptors (PRRs) recognize conserved pathogen- or microbe-associated molecular patterns (PAMPs or

MAMPs), such as fungal chitin or bacterial flagellin, to activate PTI (Jones & Dangl, 2006). PRRs include receptor-like kinases (RLKs) or receptor-like proteins (RLPs) with different extracellular domains that recognize the pathogen, providing basal immunity to most nonadapted pathogens (Ngou et al., 2022). However, effector molecules are delivered by pathogens to avoid or suppress PTI (Toruño et al., 2016). ETI is mediated by cytoplasmic nucleotide-binding (NB) domain-leucine-rich repeat (LRR) containing receptors (NLRs), which play important roles in plant innate immunity (Macho & Zipfel, 2014; Ngou et al., 2022). Understanding the molecular mechanisms underlying plant immune defence to *Diaporthe* spp. is central to increasing soybean resistance to SSC.

Since the early 1990s, germplasm screening and molecular approaches aiming to identify SSC resistance sources have been performed, leading to the development of soybean SSC-resistant cultivars (Table 2) (Brumer et al., 2018; Chiesa et al., 2009; Keeling, 1982; Mengistu et al., 2007; Vidić et al., 2013). The first four major loci for resistance to *D. aspalathi* in soybean were named *Rdc1* to *Rdc4* (Bowers et al., 1993; Kilen & Hartwig, 1987; Tyler, 1996). However, these loci conferred resistance to *D. aspalathi* but not to *D. caulivora* and were renamed *Rdm1–4* (Pioli et al., 2003). Chiesa et al. (2009) demonstrated the existence of a complex genomic region leading to resistance to isolates of *D. aspalathi* in the Hutcheson soybean cultivar, with at least two closely located resistance loci on chromosome 8, *Rdm4* and *Rdm5*. A sixth resistance locus was found in PI 398469 and was named as *Rdc?* (*Rdm?*) (Shearin, 2011). More recently, *RdmMJ19RR* was identified as a new resistance locus to SSC caused by *D. aspalathi*, located on chromosome 6 in cultivar MJ19RR (Gilli et al., 2020). Both resistance loci, *Rdm3* in both the cultivar Crockett and PI 398469 and *Rdm?* in PI 398469, are located on chromosome 14 and appear to be clustered with several genes encoding PRRs and NLRs (Maldonado dos Santos et al., 2019; Menke et al., 2023; Shearin, 2011). Several of these resistance loci have been mapped less than 14 cM from simple-sequence repeats (SSRs) or single-nucleotide polymorphism (SNP) markers that may be suitable for marker-assisted selection in breeding programmes (Chiesa et al., 2009; Gilli et al., 2020; Shearin, 2011).

By performing a genome-wide association study (GWAS), using phenotypic data of a USDA soybean germplasm collection and public SNPs data, two SNPs associated with SSC caused by *D. caulivora* (ss715617869) and *D. aspalathi* (ss715617951) were identified in a region on chromosome 14 (Chang et al., 2016). Although SNPs were located within genes encoding a heat shock protein 70 (HSP70) and an unknown protein, the occurrence of two LRR-RLK- and two NBS-LRR-encoding genes located between ss715617869 and ss715617951 suggests their possible involvement in plant resistance. More recently, Maldonado dos Santos et al. (2019) identified marker loci associated with *D. aspalathi* resistance through GWAS using a panel of 295 accessions from different regions of the world, including important Brazilian elite cultivars. This study revealed 19 SNPs on the same region of chromosome 14 associated with resistance to *D. aspalathi*. Two of these SNPs were located in genes encoding a leucine-rich repeat receptor-like kinase (Glyma.14G026300) and a Toll/interleukin-1 receptor homology (TIR) domain-containing NLR (Glyma.14G024400), which could play important functions in plant immunity. Other significant SNPs associated with SSC resistance were identified in Glyma.14G024300, a DEAD/DEAH box RNA helicase, Glyma.14G026700, a serine–threonine protein kinase and Glyma.14G026500, a protein tyrosine kinase (Maldonado dos Santos et al., 2019).

In Argentina, different sources of resistance to *D. caulivora* were found (Pioli et al., 2012), and the first resistance locus for *D. caulivora*, named *Rdc1*, was recently identified (Peruzzo et al., 2019). Additionally, resistance sources for *D. longicolla* were found in different plant introduction (PI) and breeding lines (Jackson et al., 2005; Minor et al., 1993; Smith et al., 2008; Zimmerman & Minor, 1993). Genetic data from these resistant genotypes show that one or two dominant resistance loci, present on chromosomes 13 and 14, confer resistance to Phomopsis seed decay (PSD), although loci names have not been assigned yet (Jackson et al., 2005; Minor et al., 1993; Ploper et al., 1992; Roy & Abney, 1988; Zimmerman & Minor, 1993). Moreover, two QTLs conferring resistance to PSD were identified, PSD-10–2 on chromosome 10 and PSD 6–1 on chromosome 6 (Sun et al., 2013). Interestingly, several QTLs associated with resistance to other fungal diseases are located in proximity to the molecular markers associated with PSD resistance (Sun et al., 2013).

Transcriptional profiling is a powerful tool to unravel key mechanisms involved in defence activation and resistance against different *Diaporthe* species causing SSC. Expression studies of a limited number of defence genes have shown that genes encoding a pathogenesis-related (PR) protein-1 (PR-1), β-1,3-glucanase (PR-2), chitinases (PR-3 and PR-4), osmotin-like protein (PR-5), peroxidase (PR-9), PR-10, lipoxygenases, a phenylalanine-ammonia lyase and a chalcone synthase are induced in *D. caulivora*- and *D. aspalathi*-inoculated soybean plants (Mena et al., 2020; Upchurch & Ramirez, 2010). Recently, the first plant transcriptomes of soybean tissues infected with a *Diaporthe* species causing SSC, in this case *D. caulivora*, have been reported (Mena et al., 2023). Comparison of transcription profiles between untreated Génesis 5601 genotype (resistant to *D. caulivora*) and Williams genotype (susceptible to *D. caulivora*) highlighted increased basal transcript levels of several genes involved in defence, such as *PRR*, *NLR* and *PR* genes, in resistant compared with susceptible plants. Furthermore, during the early stages of *D. caulivora* infection (8 hpi), Génesis 5601 increased the expression levels of 1028 genes, many of them with defensive functions, while at this same time point Williams only increased expression of 434 genes. These findings suggest that higher basal expression levels, as well as earlier expression of defence-related genes in Génesis 5601, contribute to SSC resistance in this cultivar. At a later time point of infection, 48 hpi, the number of induced defence genes was similar in both genotypes, suggesting that the first hours of infection are fundamental to define the outcome of the disease. Resistance to *D. caulivora* seems to rely on defence activation through perception of

TABLE 2 Soybean loci conferring resistance to stem canker.

Causal agent	Locus	Chromosome	Marker position[a]		References
			cM	bp	
Diaporthe aspalathi	*Rdm1*	–	–	–	Kilen and Hartwig (1987)
	Rdm2	–	–	–	
	Rdm3	–	–	–	Bowers et al. (1993)
	Rdm4	8	–	–	Bowers et al. (1993), Tyler (1996)
	Rdm5	8	12.4	–	Chiesa et al. (2009)
	Rdm?	14	–	–	Shearin, 2011
	RdmMJ19RR	6	13.3	47,516,523	Gilli et al. (2020)
	GBSRdm370	14	–	1,744,370	Maldonado dos Santos et al. (2019)
	GBSRdm556		–	1,725,556	
	GBSRdm287		–	1,710,287	
	GBSRdm224		–	1,986,224	
	GBSRdm562		–	1,740,562	
	GBSRdm793		–	1,768,793	
	GBSRdm339		–	1,921,339	
	GBSRdm374		–	1,921,374	
	GBSRdm219		–	1,795,219	
	GBSRdm204		–	1,751,204	
	GBSRdm516		–	1,612,516	
	GBSRdm964		–	1,850,964	
	GBSRdm114		–	1,851,114	
	GBSRdm450		–	1,612,450	
	GBSRdm397		–	1,612,397	
	GBSRdm518		–	1,744,518	
	GBSRdm120		–	1,741,120	
	GBSRdm712		–	1,581,712	
	GBSRdm875		–	1,581,875	
Diaporthe caulivora	*Rdc1*	13	–	–	Peruzzo et al. (2019)
Diaporthe longicolla	PSD 6–1	6	110.8	31,490,622–44,049,891	Sun et al. (2013)
	PSD-10–2	10	85.8	46,052,103–46,657,863	
	–	13	5.9–12.7	32,196,800	Jackson et al. (2005), Minor et al. (1993), Ploper et al. (1992), Roy and Abney (1988), Zimmerman and Minor (1993)
	–	14	4.3–15.8	971,657–2,956,930	

[a]Marker position (bp) based on the *Glycine max* genome assembly version Gmax2.0, only starting position is shown for SSR markers.

the pathogen by PRRs and NLRs, transcriptional reprogramming, biosynthesis of hormones and phenylpropanoids, production of small heat shock proteins and PR genes (Mena et al., 2023).

4.2 | Genomics and SSC management

Recent comparative genomics studies of *Diaporthe* spp. causing SSC have revealed new sources of variable genomic regions for identification, diagnosis and discrimination of *Diaporthe* spp. and isolates with different aggressiveness (Muterko et al., 2023). A simple tandem repeat containing a motif with a consensus DNA sequence specific for *D. caulivora* D57 was identified (Muterko et al., 2023). This motif is absent in the genomes of other *Diaporthe* spp. and Ascomycota, making this sequence a promising candidate for species-specific diagnostic DNA markers. A recently developed quadruplex real-time PCR assay based on *EF1-α* sequences specifically differentiates between *D. longicolla*, *D. caulivora*, *D. eres* and *D. novem*, allowing effective detection and quantification of *Diaporthe* spp. from different soybean samples relevant for disease control (Hosseini et al., 2021). Next-generation sequencing technologies confer new opportunities for advanced genomic-based diagnostics of *Diaporthe* spp. and give valuable information of the fungal species present in canker lesions, as well as ideal timing

for chemical applications in susceptible cultivars. A metagenomic sequencing approach based on Nanopore long reads technology and Illumina was recently used for detection and identification of the boxwood pathogen *Calonectria pseudonaviculata* at strain-level resolution (Yang et al., 2022). Successful applications of metagenomics for fungal pathogen diagnosis have been used in different crops and will become a more common practice in disease management (Gökdemir et al., 2022).

The availability of efficient transformation methods for *D. caulivora* and *D. longicolla* (Montoya et al., 2021; Zhang et al., 2023), together with genomic analysis, allows the identification and functional validation of fungal genes associated with pathogenicity. This will generate a more comprehensive understanding of the pathogen infection strategies and the molecular mechanisms employed to counteract the plant immune system. Future research focused on *Diaporthe* candidate effectors, including core effector proteins and species-specific candidate effectors, employing soybean inoculation assays, or transient expression analyses in *Nicotiana benthamiana*, will certainly advance our knowledge of their functions and possible applications in disease management. Effectoromics was applied in functional studies of candidate effectors of the soybean rust fungus *Phakopsora pachyrhizi*, showing that this approach could accelerate the search for NLR-mediated resistance through screening for immune activation or suppression phenotypes (Qi et al., 2018). The identification of soybean-resistant sources based on the virulence of *Diaporthe* isolates and SSC development in different soybean cultivars will contribute to the understanding of pathogenic variability and the selection of resistant soybean lines. The presence of SNPs in defence genes associated with SSC resistance, together with the identification of NLRs, RLKs and other immune-related genes differentially expressed in contrasting soybean genotypes (Maldonado dos Santos et al., 2019; Mena et al., 2023), provides promising candidate genes for functional validation. The knowledge generated will give novel insights into resistant mechanisms to *Diaporthe* spp., and benefit breeding programmes for SSC resistance.

5 | CONCLUSIONS

SSC is a disease that involves several *Diaporthe* spp., and during recent years, the use of resistant cultivars to *D. aspalathi* correlates with the presence of other *Diaporthe* spp., including *D. caulivora* and *D. longicolla*, among others. A deeper understanding of the molecular mechanisms involved in the different soybean–*Diaporthe* spp. interactions is required to develop effective and more durable strategies for plant disease prevention. The development of precise diagnostic tools for different *Diaporthe* spp., combined with field monitoring, will further enhance our understanding of fungal dynamics and host ranges. Future studies in selected candidate effectors and resistance genes will increase our knowledge on the molecular basis of SCC resistance and benefit application in plant disease management through new technologies like genome editing.

ACKNOWLEDGEMENTS

The authors thank Dr Pablo Enrique Grijalba (Universidad de Buenos Aires, UBA, Department of Plant Production, Argentina), for kindly providing us with the *D. aspalathi* isolate Dpm1. This work was funded by 'Programa de Desarrollo de las Ciencias Básicas (PEDECIBA)' Uruguay and 'Programa para Grupo de I + D Comisión Sectorial de Investigación Científica, Universidad de la República', Uruguay.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed.

ORCID

Eilyn Mena https://orcid.org/0000-0002-7957-8977
Inés Ponce de León https://orcid.org/0000-0001-6495-4055

REFERENCES

Abdelmagid, A., Hafez, M., Lawley, Y., Adam, L.R. & Daayf, F. (2019) First report of northern stem canker caused by *Diaporthe caulivora* on soybean in western Canada. *Plant Disease*, 103, 372.

Asante, S.N.A., Wolffhechel, H. & Neergaard, E. (1998) First report of *Diaporthe phaseolorum* var. *meridionalis* on soybean seeds from Ghana. *Plant Disease*, 82, 1401.

Athow, K.L. & Caldwell, R.M. (1954) A comparative study of *Diaporthe* stem canker and pod and stem blight of soybeans. *Phytopathology*, 44, 319–325.

Backman, P.A., Crawford, M.A., White, J., Thurlow, D.L. & Smith, L.A. (1981) Soybean stem canker: a serious disease in Alabama. *Highlights of Agricultural Research*, 28, 6.

Backman, P.A., Weaver, D.B. & Morgan-Jones, G. (1985) Soybean stem canker: an emerging disease problem. *Plant Disease*, *69*, 641–648.

Baker, D.M. & Minor, H.C. (1987) Infection of immature soybean pods and seeds by *Phomopsis longicolla*. *Canadian Journal of Microbiology*, 33, 797–801.

Baumgartner, K., Fujiyoshi, P.T., Travadon, R., Castlebury, L.A., Wilcox, W.F. & Rolshausen, P.E. (2013) Characterization of species of *Diaporthe* from wood cankers of grape in eastern North American vineyards. *Plant Disease*, 97, 912–920.

Benavidez, R., Pioli, R.N. & Morandi, E.N. (2010) Response of the edamame edible soybean germplasm to *Diaporthe phaseolorum*, causal agent of soybean stem canker, in Argentina. *Tropical Plant Pathology*, 35, 48–51.

Bertolini, P. & Tanzi, M. (1987) On the pathogenic mycoflora of soybean seeds. *Informatore Fitopatologico*, 37, 39–42.

Bowers, G.R., Ngeleka, K. & Smith, O.D. (1993) Inheritance of stem canker resistance in soybean cultivars Crockett and Dowling. *Crop Science*, 33, 67–70.

Bowers, G.R. & Russin, J.S. (1999) Soybean disease management. In: Heatherly, L.G. & Hodges, H.F. (Eds.) *Soybean production in the mid-south*. Boca Raton, FL: CRC Press, pp. 231–272.

Brumer, B.B., Lopes-Caitar, V.S., Chicowski, A.S., Beloti, J.D., Castanho, F.M., Gregório da Silva, D.C. et al. (2018) Morphological and molecular characterization of *Diaporthe* (anamorph *Phomopsis*) complex and pathogenicity of *Diaporthe aspalathi* isolates causing stem canker in soybean. *European Journal of Plant Pathology*, 151, 1009–1025.

Campbell, M.A., Li, Z. & Buck, J.M. (2017) Development of southern stem canker disease on soybean seedlings in the greenhouse using a modified toothpick inoculation assay. *Crop Protection*, 100, 57–64.

Cao, L., Luo, D., Lin, W., Yang, Q. & Deng, X. (2022) Four new species of *Diaporthe* (*Diaporthaceae*, *Diaporthales*) from forest plants in China. *MycoKeys*, 91, 25–47.

Chang, H., Lipka, A.E., Domier, L.L. & Hartman, G.L. (2016) Characterization of disease resistance loci in the USDA soybean germplasm collection using genome wide association studies. *Genetics and Resistance*, 106, 1139–1151.

Chen, Y., Zhou, Q., Sun, Y., Li, R., Liu, Y. & Zhou, T. (2020) First report of a postharvest fruit rot on apple caused by *Diaporthe phaseolorum* var. *caulivora* in China: a note. *American Journal of Plant Sciences*, 11, 1612–1617.

Chiesa, M.A., Pioli, R.N. & Morandi, E.N. (2009) Specific resistance to soybean stem canker conferred by the *Rdm4* locus. *Plant Pathology*, 58, 1032–1038.

Costamilan, L.M., Yorinori, J.T., Almeida, A.M.R., Seixas, C.D.S., Binneck, E., Araújo, M.R. et al. (2008) First report of *Diaporthe phaseolorum* var. *caulivora* infecting soybean plants in Brazil. *Tropical Plant Pathology*, 33, 381–385.

Crall, J.M. (1950) Soybean diseases in Iowa in 1949. *Plant Disease Repository*, 34, 96–97.

Cui, Y.L., Duan, C.X., Wang, X.M., Li, H.J. & Zhu, Z.D. (2009) First report of *Phomopsis longicolla* causing soybean stem blight in China. *Plant Pathology*, 58, 799.

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

EPPO. (2021) *EPPO global database: Diaporthe caulivora*. Paris, France: EPPO. Available from: https://gd.eppo.int/taxon/DIAPPC/distribution [Accessed 31st March 2023].

FAOSTAT. (2023) *Food and agriculture Organization of the United Nations*. FAOSTAT Data of Crops. Available from: https://www.fao.org/faostat/en/#data/QCL [Accessed 27th September 2023].

Fehr, W.R., Caviness, C.E., Burmood, D.T. & Pennington, J.S. (1971) Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. *Crop Science*, 11, 929–931.

Fernández, F.A. & Hanlin, R.T. (1996) Morphological and RAPD analyses of *Diaporthe phaseolorum* from soybean. *Mycologia*, 88, 425–440.

Fernández, F.A., Philips, D.V., Russin, J.S. & Rupe, J.C. (1999) *Diaporthe–Phomopsis* complex. In: Hartman, G.L., Sinclair, J.B. & Rupe, J.C. (Eds.) *Compendium of soybean diseases*, 4th edition. St Paul, MN: APS Press, pp. 33–35.

Floyd, C.M. & Malvick, D.K. (2022) *Diaporthe* species associated with symptomatic and asymptomatic infection of soybean stems in Minnesota: identity, virulence, and growth characteristics. *Canadian Journal of Plant Pathology*, 44, 858–873.

Freitas, M.A., Café-Filho, A.C. & Nasser, L.C.B. (2002) Cultural practices and genetic resistance as factors affecting soybean stem canker and plant yield in the Cerrado. *Fitopatologia Brasileira*, 27, 5–11.

Gebreil, A., Micijevic, A., Weber, A., Hyronimus, L. & Mathew, F. (2015) Characterization of *Diaporthe* species infecting soybeans (*Glycine max* L.) in South Dakota. *Proceedings of the South Dakota Academy of Science*, 94, 362.

Ghimire, K., Petrović, K., Kontz, B.J., Bradley, C.A., Chilvers, M.I., Mueller, D.S. et al. (2019) Inoculation method impacts symptom development associated with *Diaporthe aspalathi*, *D. caulivora*, and *D. longicolla* on soybean (*Glycine max*). *Plant Disease*, 103, 677–684.

Gilli, J.R., Vellicce, G.R. & Bernardi, C.N. (2020) SSR markers linked to stem canker resistance in soybean *Glycine max*. *Revista de la Facultad de Ciencias Agrarias UNCuyo*, 52, 26–39.

Gökdemir, F.Ş., İşeri, Ö.D., Sharma, A., Achar, P.N. & Eyidoğan, F. (2022) Metagenomics next generation sequencing (mNGS): an exciting tool for early and accurate diagnostic of fungal pathogens in plants. *Journal of Fungi*, 8, 1195.

Gomes, R.R., Glienke, C., Videira, S.I., Lombard, L., Groenewald, J.Z. & Crous, P.W. (2013) *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia*, 31, 1–41.

Grijalba, P. & Ridao, A.C. (2012) Survival of *Diaporthe phaseolorum* var. *caulivora* (causal agent of soybean stem canker) artificially inoculated in different crop residues. *Tropical Plant Pathology*, 37, 271–274.

Harrington, T.C., Steimel, J., Workneh, F. & Yang, X.B. (2000) Molecular identification of fungi associated with vascular discoloration of soybean in the north Central United States. *Plant Disease*, 84, 83–89.

Hilário, S., Santos, L. & Alves, A. (2021) Diversity and pathogenicity of *Diaporthe* species revealed from a survey of blueberry orchards in Portugal. *Agriculture*, 11, 1271.

Hildebrand, A.A. (1956) Observations on stem canker and pod and stem blight of soybeans in Ontario. *Canadian Journal of Botany*, 34, 577–599.

Hilty, J.W. (1991) The role of soybean debris in survival and inoculum production of *Diaporthe phaseolorum* var. *caulivora*, the cause of stem canker on soybean. *Journal of the Tennessee Academy of Science*, 66, 99–101.

Hissek, K., Plenk, A. & Bedlan, G. (2017) Untersuchungen zum Vorkommen pathogener Pilze an Sojabohnen in Österreich. *Journal für Kulturpflanzen*, 69, 255–263.

Hosseini, B., El-Hasan, A., Link, T. & Voegele, R.T. (2020) Analysis of the species spectrum of the *Diaporthe/Phomopsis* complex in European soybean seeds. *Mycological Progress*, 19, 455–469.

Hosseini, B., Voegele, R.T. & Link, T.I. (2021) Establishment of a quadruplex real-time PCR assay to distinguish the fungal pathogens *Diaporthe longicolla*, *D. caulivora*, *D. eres*, and *D. novem* on soybean. *PLoS One*, 16, e0257225.

Huang, L., Zhang, X., Yang, Y., Zou, H., Fang, B. & Liu, W. (2021) High-quality genome resource of *Diaporthe destruens* causing foot rot disease of sweet potato. *Plant Disease*, 105, 3279–3281.

Hwang, C.S. & Kolattukudy, P.E. (1995) Isolation and characterization of genes expressed uniquely during appressorium formation by *Colletotrichum gloeosporioides* conidia induced by the host surface wax. *Molecular and General Genetics*, 247, 282–294.

Ivancovich, A. & Botta, G. (2003) *Enfermedades de fin de ciclo de la soja*. Información Técnica de Cultivos de Verano, campaña 2003. Publicación miscelánea INTA EEA Rafaela 100.

Jaccoud-Filho, D.S., Lee, D., Reeves, J.C., Yorinori, J.T. & Hutchins, J.D. (1997) Characterization of the seedborne fungal pathogen *Phomopsis phaseoli* f. sp. *meridionalis*: the agent of soybean stem canker. In: Hutchins, J.D. & Reeves, J.C. (Eds.) *Seed health testing: Progress towards the 21st century*. Wallingford: CAB International, pp. 147–152.

Jackson, E.W., Fenn, P. & Chen, P. (2005) Inheritance of resistance to Phomopsis seed decay in soybean PI 80837 and MO/PSD-0259 (PI 562694). *Crop Science*, 45, 2400–2404.

Jones, J.D. & Dangl, J.L. (2006) The plant immune system. *Nature*, 444, 323–329.

Keeling, B.L. (1982) A seedling test for resistance to soybean stem canker caused by *Diaporthe phaseolorum* var *caulivora*. *Phytopathology*, 72, 807–809.

Keeling, B.L. (1985) Soybean cultivar reactions to soybean stem canker caused by *Diaporthe phaseolorum* var. *caulivora* and pathogenic variation among isolates. *Plant Disease*, 69, 132–133.

Keeling, B.L. (1988) Influence of temperature on growth and pathogenicity of geographic isolates of *Diaporthe phaseolorum* var. *caulivora*. *Plant Disease*, 72, 220–222.

Kilen, T.C. & Hartwig, E.E. (1987) Identification of single genes controlling resistance to stem canker in soybean. *Crop Science*, 27, 220–222.

Kmetz, K.T., Schmitthenner, A.F. & Ellett, C.W. (1978) Soybean seed decay: prevalence of infection and symptom expression caused by *Phomopsis* sp., *Diaporthe phaseolorum* var. *sojae*, and *D. phaseolorum* var. *caulivora*. *Phytopathology*, 68, 836–840.

Krausz, J.P. & Fortnum, B.A. (1983) An epiphytotic of *Diaporthe* stem canker of soybean in South Carolina. *Plant Disease*, 67, 1128–1129.

Kulik, M.M. (1988) Observations by scanning electron and bright-field microscopy on the mode of penetration of soybean seedlings of *Phomopsis phaseoli*. *Plant Disease*, 72, 115–118.

Lalitha, B., Snow, J.P. & Berggren, G.T. (1989) Phytotoxin production by *Diaporthe phaseolorum* var *caulivora* the causal organism of stem canker of soybean. *Phytopathology*, 79, 499–504.

Li, S., Darwish, O., Alkharouf, N., Matthews, B., Ji, P., Domier, L.L. et al. (2015) Draft genome sequence of *Phomopsis longicolla* isolate MSPL 10-6. *Genomics Data*, 3, 55–56.

Li, S., Darwish, O., Alkharouf, N.W., Musungu, B. & Matthews, B.F. (2017) Analysis of the genome sequence of *Phomopsis longicolla*: a fungal pathogen causing Phomopsis seed decay in soybean. *BMC Genomics*, 18, 688.

Li, S., Hartman, G.L. & Boykin, D.L. (2010) Aggressiveness of *Phomopsis longicolla* and other *Phomopsis* spp. on soybean. *Plant Disease*, 94, 1035–1040.

Li, S., Musungu, B., Lightfoot, D. & Ji, P. (2018) The interactomic analysis reveals pathogenic protein networks in *Phomopsis longicolla* underlying seed decay of soybean. *Frontiers in Genetics*, *9*, 104.

Li, S., Song, Q., Ji, P. & Cregan, P. (2015) Draft genome sequence of *Phomopsis longicolla* type strain TWH P74, a fungus causing *Phomopsis* seed decay in soybean. *Genome Announcements*, 3, 15.

Li, S., Song, Q., Martins, A.M. & Cregan, P. (2016) Draft genome sequence of *Diaporthe aspalathi* isolate MS-SSC91, a fungus causing stem canker in soybean. *Genomics Data*, 7, 262–263.

Lin, F., Chhapekar, S.S., Vieira, C.C., Da Silva, M.P., Rojas, A., Lee, D. et al. (2022) Breeding for disease resistance in soybean: a global perspective. *Theoretical and Applied Genetics*, 135, 3773–3872.

López-Cardona, N., Guevara-Castro, A., Gañán-Betancur, L. & Amaya-Gómez, C.V. (2021) First report of *Diaporthe ueckerae* causing stem canker on soybean (*Glycine max*) in Colombia. *Plant Disease*, 105, 4162.

Lu, X., Robertson, A.E., Byamukama, E.Z. & Nutter, F.W., Jr. (2010) Evaluating the importance of stem canker of soybean in Iowa. *Plant Disease*, 94, 167–173.

Macho, A.P. & Zipfel, C. (2014) Plant PRRs and the activation of innate immune signaling. *Molecular Cell*, 54, 263–272.

Maldonado dos Santos, J.V., Capote, E.G., de Lima, A.L., Bley, B., Brombini, A., Moreira, R. et al. (2019) Association mapping of a locus that confers southern stem canker resistance in soybean and SNP marker development. *BMC Genomics*, 20, 798.

Maldonado dos Santos, J.V., Sant'Ana, G.C., Wysmierski, P.T., Todeschini, M.H., Garcia, A. & Meda, A.R. (2022) Genetic relationships and genome selection signatures between soybean cultivars from Brazil and United States after decades of breeding. *Scientific Reports*, 12, 10663.

Mathew, F.M., Alananbeh, K.M., Jordahl, J.G., Meyer, S.M., Castlebury, L.A., Gulya, T.J. et al. (2015) Phomopsis stem canker: a reemerging threat to sunflower (*Helianthus annuus*) in the United States. *Phytopathology*, 105, 990–997.

Mathew, F.M., Castlebury, L.A., Alananbeh, K., Jordahl, J.G., Taylor, C.A., Meyer, S.M. et al. (2015) Identification of *Diaporthe longicolla* on dry edible pea, dry edible bean, and soybean in North Dakota. *Plant Health Progress*, 16, 71–72.

Mathew, F.M., Gulya, T.J., Jordahl, J.G. & Markell, S.G. (2018) First report of stem disease of soybean (*Glycine max*) caused by *Diaporthe gulyae* in North Dakota. *Plant Disease*, 102, 240.

Mena, E., Garaycochea, S., Stewart, S., Montesano, M. & Ponce de León, I. (2022) Comparative genomics of plant pathogenic *Diaporthe* species and transcriptomics of *Diaporthe caulivora* during host infection reveal insights into pathogenic strategies of the genus. *BMC Genomics*, 23, 175.

Mena, E., Reboledo, G., Stewart, S., Montesano, M. & Ponce de León, I. (2023) Comparative analysis of soybean transcriptional profiles reveals defense mechanisms involved in resistance against *Diaporthe caulivora*. *Scientific Reports*, 13, 13061.

Mena, E., Stewart, S., Montesano, M. & Ponce de León, I. (2020) Soybean stem canker caused by *Diaporthe caulivora*; pathogen diversity colonization process and plant defense activation. *Frontiers in Plant Science*, 10, 1733.

Mengistu, A., Castlebury, L., Smith, R., Ray, J. & Bellaloui, N. (2009) Seasonal progress of *Phomopsis longicolla* infection on soybean plant parts and its relationship to seed quality. *Plant Disease*, 93, 1009–1018.

Mengistu, A., Castlebury, L.A., Smith, J.R., Rossman, A.Y. & Reddy, K.N. (2007) Isolates of *Diaporthe-Phomopsis* from weeds and their effect on soybean. *Canadian Journal of Plant Pathology*, 29, 283–289.

Mengistu, A. & Heatherly, L.G. (2006) Planting date, irrigation, maturity group, year, and environment effects on *Phomopsis longicolla*, seed germination, and seed health rating of soybean in the early soybean production system of the midsouthern USA. *Crop Protection*, 25, 310–317.

Menke, E., Widyawan, M.H., Bachleda, N., Buck, J., Shearin, Z., Boerma, H.R. et al. (2023) Genetic mapping and confirmation of the *Rdm3* locus underlying resistance to southern stem canker in soybean. *Crop Science*, *63*, 164–175.

Minor, H.C., Brown, E.A., Doupnik, B., Jr., Elmore, R.W. & Zimmerman, M.S. (1993) Registration of Phomopsis seed decay resistant soybean germplasm MO/PSD-0259. *Crop Science*, 33, 1105.

Moleleki, N., Preisig, O., Wingfield, M.J., Crous, P.W. & Wingfield, B.D. (2002) PCR-RFLP and sequence data delineate three *Diaporthe* species associated with stone and pome fruit trees in South Africa. *European Journal of Plant Pathology*, 108, 909–912.

Montoya, M.R.A., Massa, G.A., Colabelli, M.N. & Ridao, A.C. (2021) Efficient *Agrobacterium tumefaciens*-mediated transformation system of *Diaporthe caulivora*. *Journal of Microbiological Methods*, 184, 106197.

Morgan-Jones, G. (1989) The *Diaporthe/Phomopsis* complex: taxonomic consideration. In: Pascale, A.J. (Ed.) *Proceedings of conference on world soybean research IV, 5–9 March*, Buenos Aires, Argentina: Edotora S.R.L. pp. 1699–1706.

Muterko, A., Surina, T. & Dobrovolskaya, O. (2023) The genome of a far eastern isolate of *Diaporthe caulivora*, a soybean fungal pathogen. *Applied Microbiology and Biotechnology*, 107, 1311–1327.

Ngou, B.P.M., Ding, P. & Jones, J.D.G. (2022) Thirty years of resistance: zig-zag through the plant immune system. *The Plant Cell*, 34, 1447–1478.

Niaz, S.I., Khan, D., Naz, R., Safdar, K., Ul Abidin, S.Z., Khan, I.U. et al. (2020) Antimicrobial and antioxidant chlorinated azaphilones from mangrove *Diaporthe perseae* sp. isolated from the stem of Chinese mangrove *Pongamia pinnata*. *Journal of Asian Natural Products Research*, 23, 1077–1084.

Nishmitha, K., Dubey, S.C. & Kamil, D. (2022) Diversity analysis of different *Diaporthe* (*Phomopsis*) species and development of molecular marker to identify quarantine important species *Phomopsis phaseolorum*. *3 Biotech*, 12, 31.

Oh, J.H. (1998) Effect of field sanitation on the pod and stem blight caused by *Phomopsis* spp. in soybean. *Korean Journal of Plant Pathology*, 14, 526–530.

Olson, T.R., Gebreil, A., Micijevic, A., Bradley, C.A., Wise, K.A., Mueller, D.S. et al. (2015) Association of *Diaporthe longicolla* with black zone lines on mature soybean plants. *Plant Health Progress*, 16, 118–122.

Peruzzo, A.M., Hernández, F.E., Pratta, G.R., Ploper, L.D. & Pioli, R.N. (2019) Identification and inheritance of an *Rdc* gene resistance to soybean stem canker (*Diaporthe phaseolorum* var. *caulivora*). *European Journal of Plant Pathology*, 154, 1179–1184.

Pioli, R.N., Cairo, C.A., Pratta, G. & Morandi, E.N. (2012) Análisis de la resistencia a *Diaporthe phaseolorum* var. *caulivora* en dos fuentes de germoplasma de *Glycine max*. In: Scharovsky, G., Zorzoli, R., Nestares, G., López, F. & Grassi, E. (Eds.) *Proceedings XV Congreso Latinoamericano de Genética, XLI Congreso Argentino de Genética, XLV Congreso de la Sociedad Genética de Chile, II Reunión Regional*

SAG-Litoral; 28–31 October 2012. Rosario, Argentina: ALAG, pp. 10–15.

Pioli, R.N., Morandi, E.N. & Bisaro, V. (2001) First report of soybean stem canker caused by *Diaporthe phaseolorum* var. *caulivora* in Argentina. *Plant Disease*, 85, 95.

Pioli, R.N., Morandi, E.N., Luque, A. & Gosparini, C.O. (2002) Recent outbreak of soybean stem canker caused by *Diaporthe phaseolorum* var. *caulivora* in the main soybean-producing region of Argentina. *Plant Disease*, 86, 1403.

Pioli, R.N., Morandi, E.N., Martínez, M.C., Lucca, F., Tozzini, A., Bisaro, V. et al. (2003) Morphologic, molecular, and pathogenic characterization of *Diaporthe phaseolorum* variability in the core soybean-producing area of Argentina. *Phytopathology*, 93, 136–146.

Ploetz, R.C. & Shokes, F.M. (1985) Soybean stem canker incited by ascospores and conidia of the fungus causing the disease in the southeastern United States. *Plant Disease*, 69, 990–992.

Ploper, L.D., Abney, T.S. & Roy, K.W. (1992) Influence of soybean genotype on rate of seed maturation and its impact on seedborne fungi. *Plant Disease*, 76, 287–292.

Qi, M., Grayczyk, J.P., Seitz, J.M., Lee, Y., Link, T.I., Choi, D. et al. (2018) Suppression or activation of immune responses by predicted secreted proteins of the soybean rust pathogen *Phakopsora pachyrhizi*. *Molecular Plant-Microbe Interactions*, 31, 163–174.

Rossman, A.Y., Adams, G.C., Cannon, P.F., Castlebury, L.A., Crous, P.W., Gryzenhout, M. et al. (2015) Recommendations of generic names in *Diaporthales* competing for protection or use. *IMA Fungus*, 6, 145–154.

Roth, M.G., Webster, R.W., Mueller, D.S., Chilvers, M.I., Faske, T.R., Mathew, F.M. et al. (2020) Integrated management of important soybean pathogens of the United States in changing climate. *Journal of Integrated Pest Management*, 11, 1–28.

Rothrock, C.S., Hobbs, T.W. & Phillips, D.V. (1985) Effects of tillage and cropping system on incidence and severity of southern stem canker of soybean. *Phytopathology*, 75, 1156–1159.

Roy, K.W. & Abney, T.S. (1988) Colonization of pods and infection of seeds by *Phomopsis longicolla* in susceptible and resistant soybean lines inoculated in the greenhouse. *Canadian Journal of Plant Pathology*, 10, 317–320.

Roy, K.W., Ratnayake, S. & McLean, K. (1997) Colonization of weeds by *Phomopsis longicolla*. *Canadian Journal of Plant Pathology*, 19, 193–196.

Ruocco, M., Baroncelli, R., Cacciola, S.O., Pane, C., Monti, M.M., Firrao, G. et al. (2018) Polyketide synthases of *Diaporthe helianthi* and involvement of DhPKS1 in virulence on sunflower. *BMC Genomics*, 19, 27–39.

Rupe, J.C. (2015) Stem Canker. In: Hartman, G.L., Rupe, J.C., Sikora, E.J., Domier, L.L., Davis, J.A. & Steffey, K.L. (Eds.) *Compendium of soybean diseases and pests*, 5th edition. St Paul, MN: APS Press, pp. 85–88.

Sánchez, M.C., Ridao, A.C. & Colavita, M.L. (2015) *Diaporthe caulivora*: agente causal de cancro del tallo predominante en cultivos de soja del sudeste bonaerense. *Revista FAVE—Ciencias Agrarias*, 14, 1–20.

Sanogo, S. & Etarock, B.F. (2009) First report of *Phomopsis longicolla* causing stem blight of Valencia peanut in New Mexico. *Plant Disease*, 93, 965.

Santos, J.M., Vrandečić, K., Cosić, J., Duvnjak, T. & Phillips, A.J. (2011) Resolving the *Diaporthe* species occurring on soybean in Croatia. *Persoonia*, 27, 9–19.

Sato, T., Viedma, L.Q., Alvarez, E., Romero, M.I. & Morel, W.P. (1993) First occurrence of soybean southern stem canker in Paraguay. *Japan Agricultural Research Quarterly*, 27, 20–26.

Shankar, M., Caowling, W.A. & Sweetingham, M.W. (1998) Histological observations of latent infection and tissue colonization by *Diaporthe toxica* in resistant and susceptible narrow-leafed lupins. *Canadian Journal of Botany*, 76, 1305–1316.

Shearin, Z. (2011) *Facilitating breeding for resistance to southern stem canker and elevated seed oleic acid content in soybean* [PhD thesis]. Georgia: University of Georgia. Electronic version available at: https://getd.libs.uga.edu/pdfs/shearin_zachary_201105_phd.pdf [Accessed 27th September 2023].

Shu, C., Chen, J., Huang, H., He, Y. & Zhou, E. (2014) First report of *Phomopsis longicolla* causing stem canker of eggplant in Guangdong, China. *Plant Disease*, 98, 426.

Silva, G.H., Teles, H.L., Zanardi, L.M., Marx Young, M.C., Eberlin, M.N., Hadad, R. et al. (2006) Cadinane sesquiterpenoids of *Phomopsis cassiae*, an endophytic fungus associated with *Cassia spectabilis* (*Leguminosae*). *Phytochemistry*, 67, 1964–1969.

Sinclair, J.B. (1999) *Diaporthe/Phomopsis* complex. In: Hartman, G., Sinclair, J.B. & Rupe, J. (Eds.) *Compendium of soybean diseases*, 4th edition. St Paul, MN: APS Press, pp. 31–32.

Smith, E.F. & Backman, P.A. (1988) Soybean stem canker: an overview. In: Wyllie, T.D. & Scott, D.H. (Eds.) *Soybean diseases of the north central region*. St Paul, MN: APS Press, pp. 47–55.

Smith, E.F. & Backman, P.A. (1989) Epidemiology of soybean stem canker in the southeastern United States: relationship between time of exposure to inoculum and disease severity. *Plant Disease*, 73, 464–468.

Smith, S., Fenn, P., Chen, P. & Jackson, E. (2008) Inheritance of resistance to Phomopsis seed decay in PI360841 soybean. *Journal of Heredity*, 99, 588–592.

Snow, J.P., Berggren, G.T., Harville, B.G. & Whitman, H.K. (1984) Stem canker: a soybean disease recently found in Louisiana. *Louisiana Agriculture Magazine*, 27, 9–24.

Stewart, S. (2015) Caracterización del agente causal del cancro del tallo de la soja en Uruguay. *Agrociencia Uruguay*, 19, 69–76.

Stovold, G.E. & Francis, A. (1987) Incidence of *Phomopsis phaseoli* (Desm.) Sacc. in crops and seed of soybean in New South Wales. *Australian Journal of Experimental Agriculture*, 27, 317–321.

Sun, S., Kim, M.Y., Van, K., Lee, Y.W., Li, B. & Lee, S.H. (2013) QTLs for resistance to Phomopsis seed decay are associated with days to maturity in soybean (*Glycine max*). *Theoretical and Applied Genetics*, 126, 2029–2038.

Sun, S., Van, K., Kim, M.Y., Min, K.H., Lee, Y.W. & Lee, S.H. (2012) *Diaporthe phaseolorum* var. *caulivora*, a causal agent for both stem canker and seed decay on soybean. *Plant Pathology Journal*, 28, 55–59.

Thompson, S.M., Tan, Y.P., Young, A.J., Neate, S.M., Aitken, E.A.B. & Shivas, R.G. (2011) Stem cankers on sunflower (*Helianthus annuus*) in Australia reveal a complex of pathogenic *Diaporthe (Phomopsis)* species. *Persoonia*, 27, 80–89.

Toruño, T.Y., Stergiopoulos, I. & Coaker, G. (2016) Plant-pathogen effectors: cellular probes interfering with plant defenses in spatial and temporal manners. *Annual Review of Phytopathology*, 54, 419–441.

Tyler, J.M. (1996) Characterization of stem canker resistance in 'Hutcheson' soybean. *Crop Science*, 36, 591–593.

Udayanga, D., Castlebury, L.A., Rossman, A.Y., Chukeatirote, E. & Hyde, K.D. (2014) Insights into the genus *Diaporthe*: phylogenetic species delimitation in the *D. eres* species complex. *Fungal Diversity*, 67, 203–229.

Udayanga, D., Xingzhong, L., McKenzie, E.H.C., Chukeatirote, E., Bahkali, A.H.A. & Hyde, K.D. (2011) The genus *Phomopsis*: biology, applications, species concepts and names of common pathogens. *Fungal Diversity*, 50, 189–225.

Upchurch, R.G. & Ramirez, M.E. (2010) Defense-related gene expression in soybean leaves and seeds inoculated with *Cercospora kikuchii* and *Diaporthe phaseolorum* var. *meridionalis*. *Physiological and Molecular Plant Pathology*, 75, 64–70.

van Niekerk, J.M., Groenewald, J.Z., Farr, D.F., Fourie, P.H., Halleen, F. & Crous, P.W. (2005) Reassessment of *Phomopsis* species on grapevines. *Australasian Plant Pathology*, 34, 27–39.

van Rensburg, J.C., Lamprecht, S.C., Groenewald, J.Z., Castlebury, L.A. & Crous, P.W. (2006) Characterisation of *Phomopsis* spp. associated

with die-back of rooibos (*Aspalathus linearis*) in South Africa. *Studies in Mycology*, 55, 65–74.
Vidić, M., Dordević, V., Petrović, K. & Miladinović, J. (2013) Review of soybean resistance to pathogens. *Ratarstvo i povrtarstvo*, 50, 52–61.
Vrandecic, K., Cosic, J., Riccion, L., Duvnjak, T. & Jurkovic, D. (2004) *Phomopsis longicolla* new pathogen on *Abutilon theophrasti* in Croatia. *Plant Pathology*, 53, 251.
Vrandecic, K., Cosic, J., Riccion, L., Duvnjak, T. & Jurkovic, D. (2005) Isolation of *Diaporthe phaseolorum* var. *caulivora* from *Abutilon theophrasti* in Croatia. *Plant Pathology*, 54, 576.
Vratarić, M., Sudarić, A., Duvnjak, T., Jurković, D. & Culek, M. (1998) The occurrence and intensity of soybean diseases in eastern Croatia. In: Scarascia, G.T., Porceddu, E. & Pagnotta, M.A. (Eds.) *Proceedings of the XV EUCARPIA congress: genetics and breeding for crop quality and resistance 20–25 September 1998*. Viterbo, Italy: Springer Science Business Media B.V, 28.
Welch, A.W. & Gilman, J.C. (1948) Heterothallic and homo-thallic types of *Diaporthe* on soybeans. *Phytopathology*, 38, 628–637.
Wrather, J.A., Anderson, T.R., Arsyad, D.M., Gai, J., Ploper, L.D., Porta-Puglia, A. et al. (1997) Soybean disease loss estimates for the top 10 soybean producing countries in 1994. *Plant Disease*, 81, 107–110.
Xu, T.C., Lu, Y.H., Wang, J.F., Song, Z.-Q., Hou, Y.G., Liu, S.S. et al. (2021) Bioactive secondary metabolites of the genus *Diaporthe* and anamorph *Phomopsis* from terrestrial and marine habitats and endophytes: 2010–2019. *Microorganisms*, 9, 217.
Xue, A.G., Morrison, M.J., Cober, E., Anderson, T.R., Rioux, S., Ablett, G.R. et al. (2007) Frequency of isolation of species of *Diaporthe* and *Phomopsis* from soybean plants in Ontario and benefits of seed treatments. *Canadian Journal of Plant Pathology*, 29, 354–364.
Yang, S., Johnson, M.A., Hansen, M.A., Bush, E., Li, S. & Vinatzer, B.A. (2022) Metagenomic sequencing for detection and identification of the boxwood blight pathogen *Calonectria pseudonaviculata*. *Scientific Reports*, 12, 1399.
Yorinori, J.T. (1990) Cancro da haste da soja. *Comunicado Técnico*, 44, 1–7.
Yorinori, J.T. (1996) Cancro da haste da soja: epidemiologia e controle. Londrina PR. Embrapa Soja. Circular Técnica no. 14.
Zhang, A.W., Riccioni, L., Pedersen, W.L., Kollipara, K.P. & Hartman, G.L. (1998) Molecular identification and phylogenetic grouping of *Diaporthe phaseolorum* and *Phomopsis longicolla* isolates from soybean. *Phytopathology*, 88, 1306–1314.
Zhang, C., Zhang, H., Zheng, X., Wang, Y. & Ye, W. (2023) Functional characterization of two cell wall integrity pathway components of the MAPK cascade in *Phomopsis longicolla*. *Molecular Plant-Microbe Interactions*, 36, 47–58.
Zhao, X., Li, K., Zheng, S., Yang, J., Chen, C., Zheng, X. et al. (2022) *Diaporthe* diversity and pathogenicity revealed from a broad survey of soybean stem blight in China. *Plant Disease*, 106, 2892–2903.
Zhao, X., Zhang, Z., Zheng, S., Ye, W., Zheng, X. & Wang, Y. (2021) Genome sequence resource of *Phomopsis longicolla* YC2-1, a fungal pathogen causing Phomopsis stem blight in soybean. *Molecular Plant-Microbe Interactions*, 34, 842–844.
Zimmerman, M.S. & Minor, H.C. (1993) Inheritance of *Phomopsis* seed decay resistance in soybean PI417479. *Crop Science*, 32, 96–100.

How to cite this article: Mena, E., Stewart, S., Montesano, M. & Ponce de León, I. (2023) Current understanding of the *Diaporthe*/*Phomopsis* complex causing soybean stem canker: A focus on molecular aspects of the interaction. *Plant Pathology*, 00, 1–16. Available from: https://doi.org/10.1111/ppa.13803