MINI REVIEW ARTICLE

Phytopathogenic oomycetes: a review focusing on *Phytophthora cinnamomi* and biotechnological approaches

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Abstract

The *Phytophthora* genus is composed, mainly, of plant pathogens. This genus belongs to the Oomycete class, also known as "pseudo-fungi", within the Chromista Kingdom. *Phytophthora* spp. is highlighted due to the significant plant diseases that they cause, which represents some of the most economically and cultural losses, such as European chestnut ink disease, which is caused by *P. cinnamomi*. Currently, there have been four genome assemblies placed at the National Center for Biotechnology Information (NCBI), although the progress to understand and elucidate the pathogenic process of *P. cinnamomi* by its genome is progressing slowly. In this review paper, we aim to report and discuss the recent findings related to *P. cinnamomi* and its genomic information. Our research is based on paper databases that reported probable functions to *P. cinnamomi* proteins using sequence alignments, bioinformatics, and biotechnology approaches. Some of these proteins studied have functions that are proposed to be involved in the asexual sporulation and zoosporogenesis leading to the host colonization and consequently associated with pathogenicity. Some remarkable genes and proteins discussed here are related to oospore development, inhibition of sporangium formation and cleavage, inhibition of flagellar assembly, blockage of cyst germination and hyphal extension, and biofilm proteins. Lastly, we report some biotechnological approaches using biological control, studies with genome sequencing of *P. cinnamomi* resistant plants, and gene silencing through RNA interference (iRNA).

Keywords Chestnut ink disease · Oomycetes · Biological control · RNA interference

Introduction

The species of the *Phytophthora* genus, which belong to the Oomycetes class, are a fungus-like group of organisms that are mainly plant pathogens and are widely spread around the world (Fig. 1). Among the hosts, forest species are the most affected [1, 2]. Indeed, this genus is highlighted due to the significant diseases that they are responsible for, which represents some of the most economically and cultural losses,

such as potato late blight caused by *P. infestans*, the black shank of tobacco by *P. nicotianae*, stem rot of soybean by *P. sojae* and, the ink disease of chestnut caused by *P. cinnamomi* [3–5].

The first known species of *P. cinnamomi* was described by Rands in 1922, the *Phytophthora cinnamomi* var. *cinnamomi* (Rands) [6]. Nevertheless, the other two varieties were described in 1993 and 2002. Kröber and Marwitz isolated and described *P. cinnamomi* var. *parvispora* in 1993 from the nursery plants *Beaucarnea* genus. The main differences between *P. cinnamomi* var. *cinnamomi* and *P. cinnamomi* var. *parvispora* are the smaller-sized chlamydospores and sporangia and the highest growth temperature of *P. cinnamomi* var. *parvispora* [7, 8]. However, in the recent years, a taxonomic re-evaluation has been proposed, stating that P. *cinnamomi* var. *cinnamomi* and *P. cinnamomi* var. *parvispora* are separated species (reviewed in SCANU et al. 2014). The study conducted by Scanu and collaborators used multi-gene phylogeny based on DNA sequences from internal transcribed spacer (ITS) and β-tubulin (nuclear gene regions) and, *cox1* and *cox2* (mitochondrial gene regions) combined

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with the study of morphological and physiological properties revealed that *Phytophthora parvispora* is a unique taxon [9]. A similar situation occurs with the *P. cinnamomi* var. *robiniae (*Ho), which was first isolated by Ho from *Robinia pseudoacacia* in China and differs from the others due to its absence of chlamydospores [10]. Nuclear and mitochondrial analysis conducted by Martin et al. support a new taxonomic classification, whereas *Phytophthora robiniae* would be a distinct species and, although the authors mentioned that a multilocus analysis of a larger number of isolated was in progress in 2014, to clarify this classification, up to date, there were no updates from these authors [11]. Notwithstanding that the three varieties are harmful to the ecosystems, *P. cinnamomi* var. *cinnamomi* is most studied and also the focus of our review.

*Castanea sativa (*Mill) or the European chestnut tree is very important for economic interests in Portugal, due to its fruits and wood exploitation. This tree is one of the leading orchards in Portugal, mainly in the mountainous regions of Trás-os-Montes. However, the infection by *P. cinnamomi* has been causing low productivity and economic losses for several years [12]. Moreover, this oomycete is also a concern in Brazil, since some regions have been reporting damages in some important crops, such as avocado trees (*Persea americana* Miller), plane trees (*Platanus acerifolia*), chive (*Allium fistulosum* L.) and lettuce (*Lactuca sativa* L.), with no efficient pathogen control methods [2, 13, 14].

Infection by *P. cinnamomi* results in wet rotting of the roots and collar of seedlings and trees in nurseries, plantations, and forests, which leads to the death of the plants [12]. The symptoms of the disease include reduced size and chlorotic leaves, thinning of the crown, and necrosis in the collar of the tree. Nevertheless, the roots are the part of the plant most infected and affected, producing a black exudate visible in the circumjacent soil. The necrosis in the main roots extends to the lateral roots and to the stem for some centimeters, which will lead to the death of the plant [12, 15, 16].

Despite the importance of pathogen control for *P. cinnamomi* due to its impact on economy and biodiversity, the existing approaches are limited, expensive and, mainly targeted to make the plant more tolerant of the infection [17]. The most successful option for controlling and eliminating *P. cinnamomi* is the treatment of plants with phosphite, but it demands repeated applications since its effectiveness declines over time [17, 18]. Nevertheless, data on long-term treatment with phosphite and its impact on the ecosystem are scarce, but it is known that in diseased habitats, phosphite treatment significantly reduced the loss of shrub cover, bare ground and sedge cover, but, it does not causes adverse/ negative impacts on species assemblages and structure [19, 20]. Other options for pathogen control are the use of the fungicide fosetyl-aluminum [21], the treatment with copper salts to improve host resistance [22] and, the extract of *Phlomis purpurea* [23]. Furthermore, according to Dunstan et al., the most effective control is host removal, followed by fumigation and fungicide application in the soil [18]. However, the emergence of advanced techniques for plant
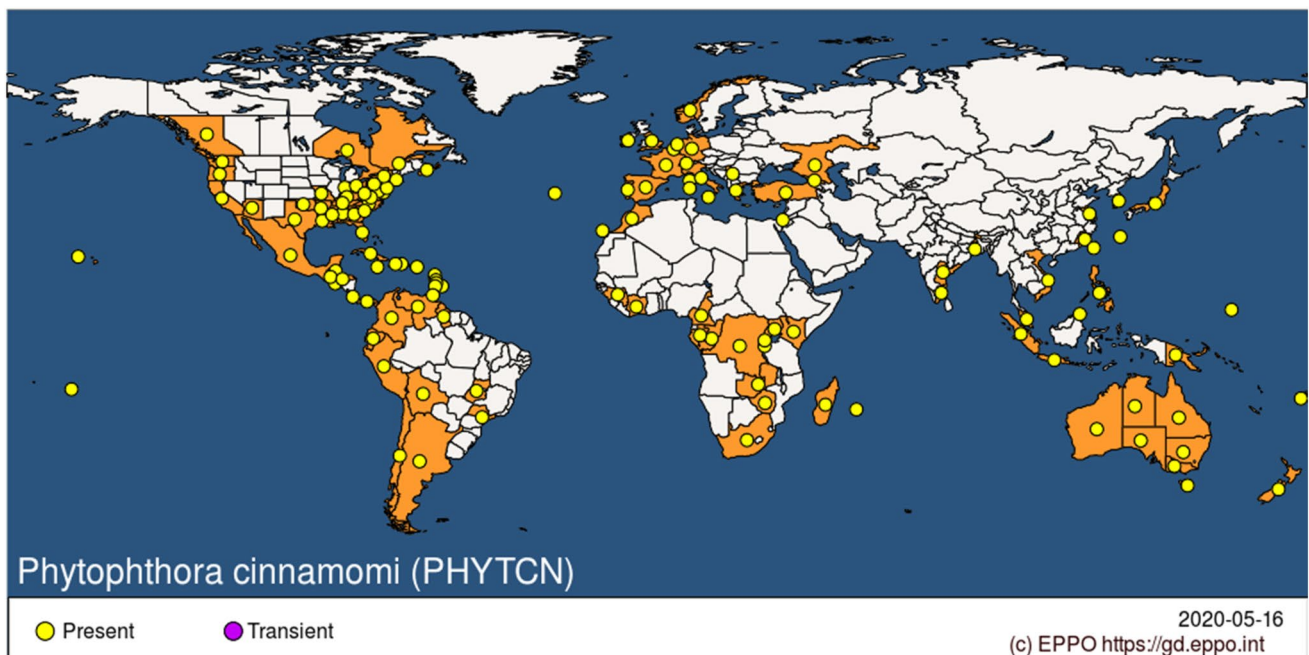

Fig. 1 Representation of geographical distribution of *Phytophthora cinnamomi* worldwide. From: European and Mediterranean Plant Protection Organization (EPPO) (2020) EPPO Global Database (available online). https://gd.eppo.int/. Access date: July 14th, 2020

breeding and pathogen control using molecular biology and biotechnology are promising tools for reducing the damage caused by *P. cinnamomi* [5].

In line with this, this review aims to discuss the mechanism of infection of *P. cinnamomi* var. *cinnamomi* with the latest advances concerning genomic information of this pathogen and the use of its genome to better understand its action and new promising findings.

Phytophthora cinnamomi strategies for plant infection

At the time of writing, the main questions concerning plant infection by *P. cinnamomi* are whether this pathogen can avoid triggering the host defence, or if it is able to suppress or overcome the host defence, or both of these actions. It is known that other species from the *Phytophthora* genus, such as *P. infestans*, *P. sojae*, and *P. capsici* have a restricted host range [24–26]. However, *P. cinnamomi* has an extensive host range and in different climate conditions, thus, another important question is which molecular factors may be responsible for this adaptation.

Phytophthora cinnamomi is a soil-borne plant pathogen that belongs to the Oomycetes class, which is a group of fungus-like microorganisms within the Kingdom Chromista [27]. This pathogen can grow in a saprophytic way, in dead organic matter, or in a parasitic way, in susceptible hosts, such as the European chestnut tree [28]. *P. cinnamomi* has sexual and asexual phases in its lifecycle (Fig. 2) and both of them are implicated in the process of host infection [16, 29].

Currently, there are four genome assemblies deposited at the National Center for Biotechnology Information (NCBI) for *P. cinnamomi* var. *cinnamomi*. These four genome assemblies (https://www.ncbi.nlm.nih.gov/genome/browse#!/eukaryotes/6958/) were obtained through Whole Genome Sequencing (WGS), using Next-Generation Sequencing (NGS) [30, 31]. The samples were from three different locations in Australia and one location in New Zealand, see Table 1 for more detailed information. The Australia ecosystems have been significant affected by *P. cinnamomi* virulence since this pathogen decimated the Jarrah forest in Western Australia and more than 40% of the plant species present in this region are susceptible to *P. cinnamomi* infection [32, 33]. However, this genomic information is not complete due to the lack of gene annotation for these genome assemblies.

Nevertheless, the use of bioinformatics tools for local alignments, such as BLAST (Basic Local Alignment Search

Fig. 2 Brief representation of the life cycle of *Phytophthora cinnamomi*. The infection of the host plant by P. cinnamomi begins through the connection of zoospores with the region of elongation of the plant roots. Then zoospores enter the encystment phase with the formation of biofilm from secreted proteins. Between 20–30 minutes after the zoospore encysting, the cysts germinate and give rise to hyphae. These hyphae are responsible for the production of enzymes that will degrade the cell wall of plants, such as those belonging to the Cell Wall-Degrading Enzymes (CWDEs) family. The intracellular and intercellular growth of hyphae in the root cortex towards the cortical and vascular tissues causes water stress and necrosis. Adapted from Hardham [33]
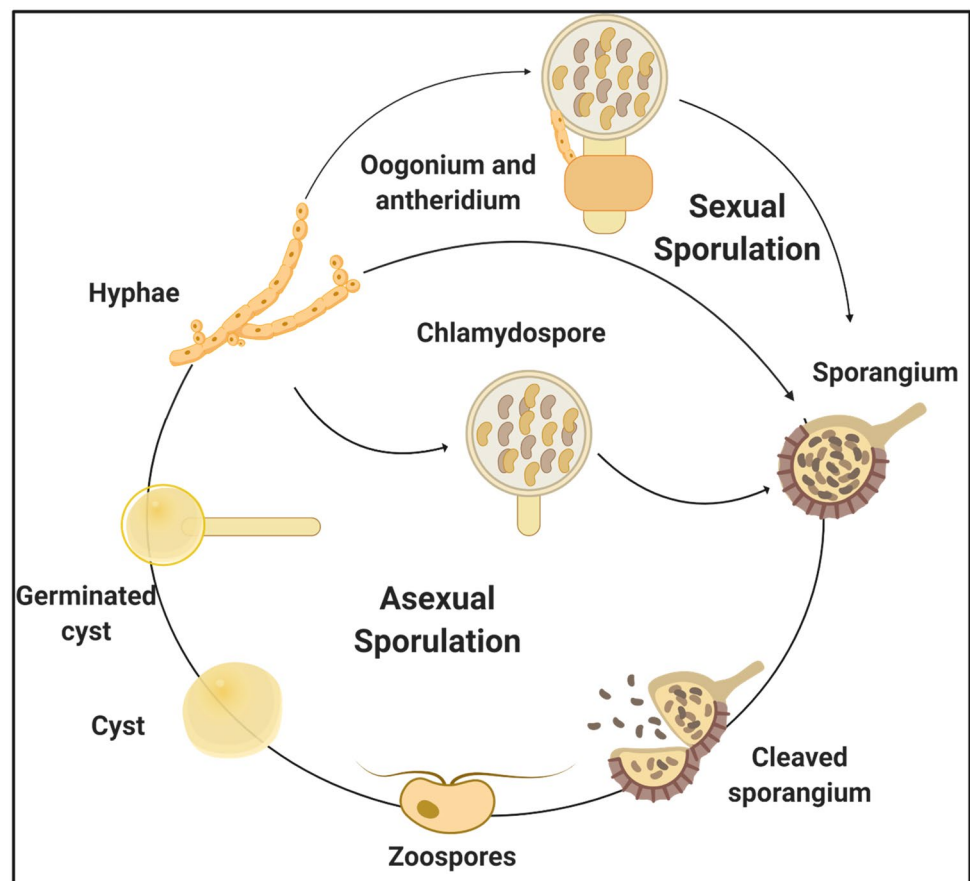
Table 1 Assemblies of different strains of *Phytophthora cinnamomi* var. *cinnamomi* placed at the National Center for Biotechnology Information (NCBI)

Organism	Strain	Substrate/ Host	Location	Year of isolation	Biosample (NCBI)	Bioproject (NCBI)	Size (Mb)	%GC	References
P. cinnamomi var. *cinnamomi*	MP94-48	*Eucalyptus marginata*	Western Australia	1994	SAMN03921829	PRJNA290836	53.69	54.00	Studholme et al. [31]
P. cinnamomi var. *cinnamomi*	NZFS 3750	*Pinus radiata*	Nelson, New Zealand	2013	SAMN03921830	PRJNA290837	53.97	54.00	Studholme et al. [31]
P. cinnamomi var. *cinnamomi*	WA94.26	*Eucalyptus marginata*	Brisbane Ranges, Australia	1994	SAMN07736482	PRJNA413098	68.06	53.20	Longmuir et al. [30]
P. cinnamomi var. *cinnamomi*	DU054	*Xanthorrhoea australis*	Southwestern Western Australia	2003	SAMN07736481	PRJNA413098	62.80	52.80	Longmuir et al. [30]

Tools) (https://blast.ncbi.nlm.nih.gov/Blast.cgi), have made it possible to propose a function for presumably some proteins of the *P. cinnamomi* genome, through the alignment with homolog sequences. All the following mentioned genes are summarized in Table 2.

During the asexual phase of the *P. cinnamomi* lifecycle sporulation and zoosporogenesis occurs, and this phase is triggered by the reduction in the availability of nutrients [33]. The asexual spores are motile and capable of forwarding movements and the resultant motile zoospores are thought to initiate the infectious process, as seen in many species of *Phytophthora* [3, 27]. Indeed, some proteins are proposed to be involved in asexual sporulation and zoosporogenesis and are also associated with the pathogenicity of *P. cinnamomi*. Gao et al. [34] reported that the silencing of the stress-associated mitogen-activated protein kinase (MAPK) gene in *P. sojae*, (*PsMPK7*), inhibited the oospore development and reduced the virulence of this pathogen; *P. cinnamomi* shares the same gene with *P. sojae*, probably with a conserved function. Similarly, Li et al. [35] showed that MAPK

Table 2 Genes of *Phytophthora cinnamomi* with probable involvement in the plant host infection, based on homology search. Adapted from Hardham et al. [16]

P. cinnamomi gene accession (FungiDB)	Homologues and gene accession (FungiDB)	Probable protein function	References
PHYCI_112968	*P. sojae* (*PsMPK7*) PHYSO_355777	Stress-associated mitogen-activated protein kinase (MAPK). **Silencing**: inhibited the oospore development and reduced the virulence.	Gao et al. [34]
PHYCI_90010	*P. sojae* (*PsMPK1*) ACJ09359	MAPK. **Silencing**: inhibited sporangium formation and reduced virulence	Li et al. [35]
PHYCI_91218	*P. infestans* (*PiGK4*) PITG_05519	G-protein-coupled receptor with a C-terminal PIP kinase domain (PiGK4). **Silencing**: inhibited the sporangial cleavage.	Hua et al. [37]
PHYCI_551329	*P. parasitica* (*PcDLC1*) ADI77080.1	Flagellar protein dynein light chain 1 (PcDLC1). **Silencing**: inhibited the flagellar assembly.	Narayan et al. [39]
PHYCI_232701	*P. sojae* (*PsHint1*) PHYSO_494520	Histidine triad domain-containing protein (PsHint1) **Silencing**: inhibited cyst germination and hyphal extension.	Zhang et al. [44]
PHYCI_327508 PHYCI_253325 PHYCI_253304	*P. parasitica* (*PPMUCL1*) PPTG_17796	Mucin-like proteins.	Larousse et al. [45]
PHYCI_93258 PHYCI_93260 PHYCI_93259 PHYCI_92597 PHYCI_323321 PHYCI_85664 PHYCI_85660 PHYCI_97296	*P. cactorum* (*Scr96*) ALC04448	Small cysteine-rich (SCR) effector proteins. **Silencing**: reduced virulence and turned more sensitive to oxidative stress.	Chen et al. [54]

is up-regulated in sporulating hyphae and early infection in *P. sojae* (*PsMPK1*) and that gene silencing revealed inhibition of sporangium formation and also reduced virulence. The sporangial cleavage is an important step in the process of infection and occurs to create uninucleate zoospores from multinucleate sporangia [36]. Hua et al. [37] reported that silencing of G-protein-coupled receptor 4 with a C-terminal PIP kinase domain in *P. infestans* (*PiGK4*), a homologue gene in *P. cinnamomi*, inhibits the sporangial cleavage and, consequently, the infection.

The zoospores formed by sporangial cleavage are the key to the infection through their active movement due to the presence of an anterior flagellum and a posterior flagellum [28]. These flagella have the substructure of a eukaryotic flagellum (9 + 2 microtubular) and the flagellar proteins are encoded for by two genes: dynein light chain 1 (*PcDL1*) and radial spoke protein 6 (*PcRSP6*) [38, 39]. Narayan et al. [39] showed that silencing the homologue *PcDLC1* gene in *P. parasitica* resulted in the inhibition of the flagellar assembly, therefore avoiding the infection of the potential host.

To achieve a successful host infection, *P. cinnamomi* zoospores move towards the elongation zone of plant roots and form a cluster on the plant root surface. Once there, the encysting zoospores secrete adhesins to attach to the root surface and also secrete three glycoproteins to form a mucilage-like biofilm that covers the cyst surface [33, 40]. The next step in the host infection process is cyst germination, after the zoospore encystment, and penetration and colonization of the plant host. The initial penetration possibly depends on the action of degradative enzymes responsible for degrading the components of the plant cell wall. The Cell Wall-Degrading Enzymes (CWDEs) families are known to be present in the genomes of *Phytophthora* spp [16, 41]. After penetration of the plant root surface, the hyphae of *P. cinnamomi* grow and followed by the invasion of the root cortex and blockage of xylem, resulting in water stress. *P. cinnamomi* may continue in a biotrophic phase and, consequently, the absence of disease symptoms, or it may turn to the necrotrophic phase; this transition is mainly influenced by the plant species and environmental conditions [42, 43].

In a study of the protein function in homologue genes, Zhang et al. [44] reported that the silencing of the *PsHint1* gene from *P. sojae*, which encodes the histidine triad domain-containing protein, results in inhibition of cyst germination and hyphal extension. Larousse et al. [45] showed that the *PPMUCL1* gene from *P. parasitica* encodes for mucin-like proteins, this gene is a homologue in the *P. cinnamomi* genome and these proteins are high-molecular-weight glycoproteins found in biofilms from cysts, and probably protect the germinated cyst against desiccation. Using techniques of gene silencing for the three genes found in *P. cinnamomi* genome may reduce its virulence and pathogenicity.

Effectors and elicitors for *P. cinnamomi* interaction with the host

Despite the importance of all the molecular factors mentioned above, the *Phytophthora* genus has in its genome three distinct biomolecules that are highlighted due to their mechanism of action and importance for host infection. Here we emphasize the CWDEs families, and the molecules involved in plant-pathogen interactions: effectors and elicitors. The colonization of the plant host by *P. cinnamomi* depends on the action of CWDEs for initial penetration. These enzymes are responsible for the degradation of components of the plant cell wall, such as cellulose, hemicellulose, and pectin [16]. Within the CWDEs, some multigene families contain one or more carbohydrate-active enzyme (CAZyme) modules, and these modules are divided in glycoside hydrolase (GH) module, auxiliary activity (AA) module, carbohydrate esterase (CE) module, polysaccharide lyase (PL) model and non-catalytic carbohydrate-binding module (CBM) [16, 41, 46–48].

An effector molecule facilitates the establishment of the infection and, consequently, the establishment of the disease. An elicitor molecule, or avirulence factor, is an effector recognized by the plant and elicits a defence response from the host. Effectors are mainly proteins, such as CWDEs, however, elicitors exist in a great diversity of molecules, such as proteins, lipids, carbohydrates, and may be a molecule resulting from the digestion of the host cell wall, referred to as pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) [16, 49–52].

Phytophthora cinnamomi effectors are classified as either attack strategies or defence strategies. Among the main identified effector proteins for attack strategies in the genome of *P. cinnamomi* are elicitins, a family of low molecular weight proteins that trigger the defence mechanisms of some plant hosts [16]. Although their biological role in the infectious process remains not fully clarified, it is known that they act as carriers of sterols. Cinnamomins are the most studied elicitin from *P. cinnamomi*, and the silencing of both the α- and β-cinnamomin gene reduces the pathogen's ability to penetrate and colonize the roots of *C. sativa* [16, 53].

Small cysteine-rich (SCR) effector proteins are also produced by *P. cinnamomi*, although their function has not been elucidated, although the silencing of the encoding SCR gene (*Scr96*) in *P. cactorum* reduced pathogen virulence and made it more sensitive to oxidative stress [54]. GP42 transglutaminase is present in the *P. cinnamomi* genome and was identified by Martins et al. [55], its main function is to catalyze the acyl transfer reaction that increases the peptide bonds resistance against proteolytic degradation.

The group of effectors named CRN crinklers is present in most of the identified species of the *Phytophthora* genus.

They are responsible for crinkling the leaves and result in host cells becoming necrotic and suppresses the plant's defence [56, 57], but more studies are needed to clarify the functions of CRN proteins, because they may be the key to understanding the ability of *P. cinnamomi* to infect a wide range of host plant species [16]. Another group of effectors that may be involved in plant cell necrosis, is the Nep1-like proteins (NLPs), which may contribute to pathogen virulence by inducing a plant defence response and/or by acting as toxins [58, 59].

The best-studied and clarified effectors proteins within the *Phytophthora* genus is the RxLR effector family. These molecules are characterized by a signal peptide followed by an N-terminal conserved Arginine – any amino-acid residue – Leucine – Arginine (RxLR) motif [60]. The most common mechanism of action of RxLR effectors is the modulation of host defense by suppression of host immunity through distinct ways [61]. One example of RxLR effector is Avr3a of *P. infestans*, that confers avirulence to strains of this pathogen, once this protein binds and stabilizes the CMPG1 (E3 ubiquitin ligase protein CMG1) to suppress BAK1/SERK3-regulated immunity during the biotrophic phase of *P. infestans* infection [62, 63]. Recently, the effector Avr3a gene was identified and characterized in silico in genomic sequences of *P. cinnamomi* deposited at NCBI by Branco and Choupina [64]. Similarly, Dai et al. identified an RxLR effector, avirulence homolog protein 87 (Avh87), in the genome of *P. cinnamomi* using bioinformatics tools. In this study, they also characterized the suppressing activity of pro-apoptotic protein BAX and elicitin protein INF1-mediated cell death using *Nicotiana benthamiana* as a model [65].

The main effectors involved in pathogen defence strategies are molecules that present a diversity of mechanisms, such as suppression, deactivation, and tolerance related to the plant defence. One of the pathogen defences is protection against reactive oxygen species (ROS) that may occur as part of the plant defence response. In line with this, the *P. cinnamomi* genome contains three superoxide dismutases (SODs) and three catalase genes, and also contains genes for the signal transduction pathways [34, 66–68].

Another important effector for defence against pathogens are inhibitors of plant endoglucanases (GIPs), which are enzymes released by the plant that degrade β-1,3-glucans, key components of *Phytophthora* spp cell wall [1]. A crucial *P. cinnamomi* defence is protease inhibitors since plant proteases are important in plant immunity because they are involved in pathogen detection, activation of defence responses, and degradation of pathogen proteins. *P. cinnamomi*, like other species of the *Phytophthora* genus, produces three families of proteases inhibitor: glucanase inhibitor proteins (GIPs), Kazal-like protease inhibitors (EPI1-4), and cystatin-like protease inhibitors (EPIC1-4) [1, 69, 70]. For a better understanding of the effectors addressed in this review, we recommend the reading of Hardham and Blackman.

How biotechnology can be useful to understand *P. cinnamomi* and find a solution to environmental problems

The technological advances of the last years promoted the use of new molecular biology and biotechnology techniques to elucidate the molecular factors involved in the pathogenicity of *P. cinnamomi*. Meyer et al. used the dual RNA-sequencing, a technique that allows the simultaneous detection of pathogen and host transcripts during infection, to better understand the interaction between *P. cinnamomi* and *Eucalyptus nitens* factors in the infectious process. The main results revealed that the *E. nitens* PR-9 gene may be a common target for the CRN effector of the pathogen since a high expression of crinkler effector of *P. cinnamomi* and a down-regulation of a PR-9 gene in *E. nitens* were found [71]. In another study, the contribution of the β-cinnamomin in the *P. cinnamomi* virulence was established by using immunodepletion tests with *Lupinus angustifolius*. Moreover, the same study revealed that β-cinnamomin is secreted at different life stages of *P. cinnamomi*, through the use of a β-cinnamomin immune-labeling [72]. Furthermore, recent studies using bioinformatics approaches, heterologous protein expression system and molecular biology techniques have characterized necrosis-inducing Phytophthora protein 1 (NPP1) elicitin and an endo-1,3-β-D-glucosidase of *P. cinnamomi* [73, 74].

Identification and diagnostic

P. cinnamomi is a soil-borne pathogen that can produce oospores in host roots and soil, which makes this oomycete a persistent and difficult pathogen to manage [28]. The current control measures are soil sanitation and crop rotation, however, *P. cinnamomi* also is resistant to the most used fungicides and oomyceticides [28, 42]. In line with this, early detection is the most important step in the management of *P. cinnamomi* diseases, and approaches that use DNA identification are the most reliable [16]. Several variations of polymerase chain reaction (PCR) are available for *P. cinnamomi* identification, the most recent proposed is a loop-mediated isothermal amplification (LAMP) of DNA using specific primers designed for a new target gene (*Pcinn100006*) developed by Dai et al. [75]. The novel assay presented higher accuracy and a shorter period, which can be considered a promising diagnostic tool when compared to the conventional PCR-based and culture-dependent assay.

Biological control

The utilization of techniques in biological-control has grown in recent years in numerous biotechnology applications. The use of other organisms to control the presence and the infection by *P. cinnamomi* has been tested and the results are promising, although more studies are needed. Bosso et al. showed that *Byssochlamys nivea* and *Scopulariopsis brumptii* in laboratory studies were able to inhibit the growth of *P. cinnamomi* and *P. cambivora* and reduce the mortality of chestnut plants [76]. Supporting the use of biological-control for *P. cinnamomi*, Méndez-Bravo et al. reported that two rhizobacteria, closely related to *Bacillus acidiceler*, were able to inhibit *P. cinnamomi* growth *in vitro* by 76%, suggesting that these bacteria could be used for biological control of oomycetes [77]. Lastly, Trzewik et al. reported a practical possibility of biological protection against *P. cinnamomi* or *Piriformospora indica*, an endomycorrhizal-like fungus, in two cultivars of rhododendron plants ('Nova Zembla' and 'Alfred') [78].

Resistant plants

It is known that a wide range of plants have resistance to *P. cinnamomi* infection, among them are *Castanea crenata* and *Castanea dentata* (see Appendix 1 [79]). In Portugal, a chestnut breeding program was initiated in 2006 to introduce the resistant genes from *C. crenata* into *C. sativa* through crossing both species [80]. However, the identification of the genes involved in the resistance of *C. crenata* are still not fully clarified. Regardless, studies have reported that *C. crenata* and *C. sativa* have the same gene expression of eight genes studied, but *C. sativa* has a lower and delayed expression of these genes when infected by *P. cinnamomi* when compared to *C. crenata*, which may be related to the sensitivity of this species to this pathogen [81]. In this context, the findings reported could be useful for the development of new strategies to control the diseases caused by *P. cinnamomi*, as an early selection of resistant genotypes, or even engineering *C. sativa* to obtain better gene promoters.

Gene silencing

Ultimately, the use of molecular biology techniques of gene silencing could be a useful tool to reduce the virulence of *P. cinnamomi* and thus decrease the effects of the disease. One of the most promising tools for gene silencing is RNA-mediated gene silencing through RNA interference (RNAi). In RNAi, the gene silencing occurs by the inhibition of RNAm translate [82]. Chahed proposed gene silencing using RNAi of a glucanase inhibitor protein (*gip* gene) from *P. cinnamomi*, which probably made the pathogen more susceptible to the plant host defence. The results were promising since the chestnuts infected by the transformed *P. cinnamomi* had a smaller percentage of wilting leaves and root necrosis [83]. Pereira also proposed a transformed *P. cinnamomi* to decrease the virulence and pathogenicity of the oomycete. In this study, the genetic construction for gene silencing using RNAi was developed for the Avr3a effector from *P. cinnamomi* [84].

Conclusions

Further research of *P. cinnamomi* will necessitate the utilization of the new techniques of molecular biology, bioinformatics, and biotechnology. The identification of key genes related to the pathogenicity, the action of the proteins encoded by these genes, and the complete mechanism of infection by *P. cinnamomi* is necessary for further advances. The development of techniques for regarding genome sequencing, exome sequencing, and gene silencing are essential tools to understand *P. cinnamomi* and also to understand the mechanisms of resistance of the resistant plants.

Compliance with ethical standards

Conflict of interest All authors declare that there is no conflict of interest in this work.

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