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PATHOGEN PROFILE

The clubroot pathogen *Plasmodiophora brassicae*: A profile update

Muhammad Asim Javed[1,2,3] | Arne Schwelm[4,5] | Nazanin Zamani-Noor[6] | Rasha Salih[1,2,3] | Marina Silvestre Vañó[1,2,3] | Jiaxu Wu[1,2,3] | Melaine González García[1,2,3] | Thies Marten Heick[7] | Chaoyu Luo[1,8] | Priyavashini Prakash[1,9] | Edel Pérez-López[1,2,3]

[1]Département de phytologie, Faculté des sciences de l'agriculture et de l'alimentation, Université Laval, Quebec City, Quebec, Canada

[2]Centre de recherche et d'innovation sur les végétaux, Université Laval, Quebec City, Quebec, Canada

[3]Institute de Biologie Intégrative et des Systèmes, Université Laval, Quebec City, Quebec, Canada

[4]Department of Plant Science, Wageningen University and Research, Wageningen, Netherlands

[5]Teagasc, Crops Research Centre, Carlow, Ireland

[6]Julius Kühn-Institute, Institute for Plant Protection in Field Crops and Grassland, Braunschweig, Germany

[7]Department of Agroecology, Crop Health, Aarhus University, Slagelse, Denmark

[8]College of Agronomy and Biotechnology, Southwest University, Chongqing, China

[9]K. S. Rangasamy College of Technology, Namakkal, India

Correspondence
Edel Pérez-López, Département de phytologie, Faculté des sciences de l'agriculture et de l'alimentation, Université Laval, Quebec City, Quebec, Canada.
Email: edel.perez-lopez@fsaa.ulaval.ca

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Abstract

Background: *Plasmodiophora brassicae* is the causal agent of clubroot disease of cruciferous plants and one of the biggest threats to the rapeseed (*Brassica napus*) and brassica vegetable industry worldwide.

Disease symptoms: In the advanced stages of clubroot disease wilting, stunting, yellowing, and redness are visible in the shoots. However, the typical symptoms of the disease are the presence of club-shaped galls in the roots of susceptible hosts that block the absorption of water and nutrients.

Host range: Members of the family Brassicaceae are the primary host of the pathogen, although some members of the family, such as *Bunias orientalis*, *Coronopus squamatus*, and *Raphanus sativus*, have been identified as being consistently resistant to *P. brassicae* isolates with variable virulence profile.

Taxonomy: Class: Phytomyxea; Order: Plasmodiophorales; Family: Plasmodiophoraceae; Genus: *Plasmodiophora*; Species: *Plasmodiophora brassicae* (Woronin, 1877).

Distribution: Clubroot disease is spread worldwide, with reports from all continents except Antarctica. To date, clubroot disease has been reported in more than 80 countries.

Pathotyping: Based on its virulence on different hosts, *P. brassicae* is classified into pathotypes or races. Five main pathotyping systems have been developed to understand the relationship between *P. brassicae* and its hosts. Nowadays, the Canadian clubroot differential is extensively used in Canada and has so far identified 36 different pathotypes based on the response of a set of 13 hosts.

Effectors and resistance: After the identification and characterization of the clubroot pathogen SABATH-type methyltransferase PbBSMT, several other effectors have been characterized. However, no avirulence gene is known, hindering the functional characterization of the five intercellular nucleotide-binding (NB) site leucine-rich-repeat (LRR) receptors (NLRs) clubroot resistance genes validated to date.

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Important Link: Canola Council of Canada is constantly updating information about clubroot and *P. brassicae* as part of their Canola Encyclopedia: https://www.canolacouncil.org/canola-encyclopedia/diseases/clubroot/.

Phytosanitary categorization: PLADBR: EPPO A2 list; Annex designation 9E.

KEYWORDS
clubroot, cruciferous crops, effectors, host resistance, pathotyping, Phytomyxea, protist

1 | INTRODUCTION

More than 10 years ago, *Plasmodiophora brassicae*, the clubroot pathogen, was identified as an emerging phytosanitary problem in Canada (Hwang, Strelkov, et al., 2012). Unfortunately, since then, the clubroot pathogen has made Canada its "forever home". While only detected in around 35,000 ha in 2010, today the pathogen has spread to more than 200,000 ha in the prairie provinces of Alberta, Saskatchewan, and Manitoba alone (Government of Alberta, 2021; Government of Manitoba, 2021; Government of Saskatchewan, 2021; Strelkov et al., 2022). However, the situation outside Canada is no different, with more than 80 countries reporting the presence of the clubroot pathogen and the disease, its alarming expansion to new fields (Figure 1), as well as substantial economic losses associated with it (Czubatka-Bieńkowska et al., 2020; Dixon, 2009; Muirhead et al., 2020; Padrón-Rodríguez et al., 2022; Říčařová et al., 2016; Zamani-Noor, 2017). The presence and spread of clubroot resistance-breaking *P. brassicae* isolates gives reason for further concerns (Strelkov et al., 2018).

Since the release of the first *Pathogen Profile* for *P. brassicae* (Hwang, Strelkov, et al., 2012), much progress has been made in understanding the biology of this enigmatic pathogen and its interaction with its host. This has included the identification and characterization of the first *P. brassicae* effector, a salicylic acid defence manipulating methyltransferase, used by the clubroot pathogen to manipulate plant immunity (Ludwig-Müller et al., 2015). Another turning point was, undoubtedly, the first assembly and annotation of the *P. brassicae* genome (Schwelm et al., 2015) of the single spore isolate (SSI) e3 (Fähling et al., 2004), which opened the door to further genome assemblies (Bi et al., 2016; Daval et al., 2019; Rolfe et al., 2016; Sedaghatkish et al., 2019). Now an updated version of the e3 genome with a nearly complete chromosome level assembly is available to the research community (Stjelja et al., 2019). Similar progress has been made in understanding the relationship of *P. brassicae* with its hosts, mainly through large-scale transcriptomics (Ciaghi et al., 2019; Irani et al., 2018; Rolfe et al., 2016; Schwelm et al., 2015). This aided the identification of several subsets of candidate protein effectors that might play a key role during primary infection (Chen et al., 2019), secondary infection (Hossain et al., 2021; Pérez-López et al., 2020), or contributing to escape host resistance (Galindo-González et al., 2021). In this review, we focus on the more than 350 studies published after 2011 (Figure 1), advancing our

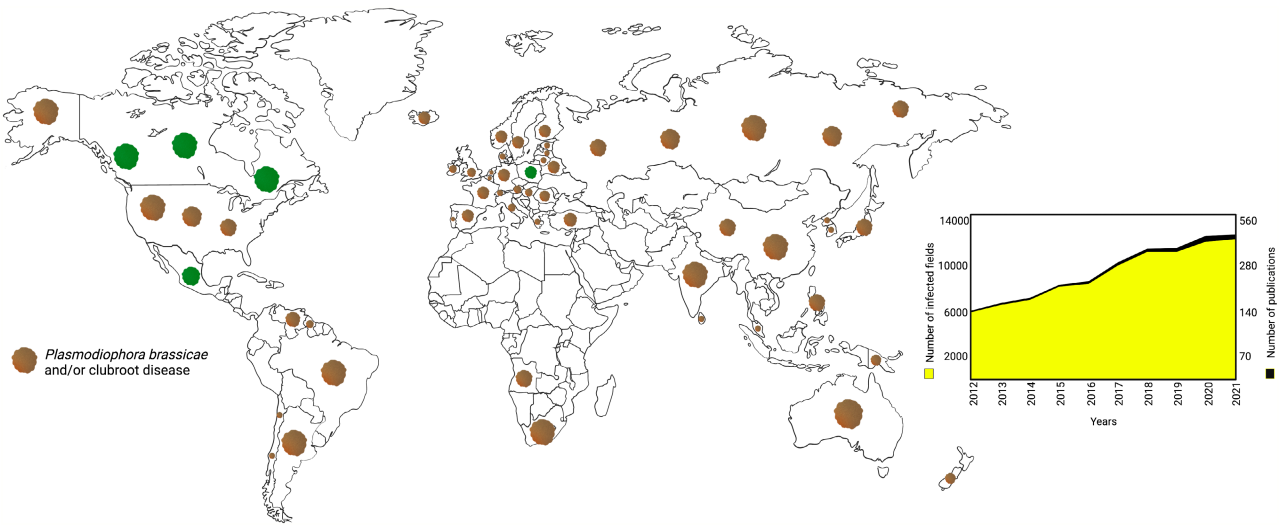

FIGURE 1 Distribution of clubroot disease and representation of the relevance of clubroot research in the last 10 years. The map shows the distribution of clubroot based on Dixon (2009). The number of infected fields in areas marked green was used to graph the number of fields infected by clubroot (Czubatka-Bieńkowska et al., 2020; Government of Alberta, 2021; Government of Manitoba, 2021; Government of Saskatchewan, 2021; Padrón-Rodríguez et al., 2022). The size of the circles reflects the size of the country. The number of clubroot-related publications from 2012 to 2021 was assessed through a Scopus search of the term clubroot or *Plasmodiophora brassicae*

understanding of the clubroot pathogen and offering suggestions of the main areas that need the attention of all "clubrooters" to keep deepening our understanding of the pathogen and the disease.

2 | THE CLUBROOT PATHOGEN

The clubroot pathogen *P. brassicae* belongs to the plasmodiophorids, a group of plant pathogenic protists (Neuhauser et al., 2014; Schwelm et al., 2017). Woronin established the genus *Plasmodiophora*, considering them as a group of Myxomycetes (Woronin, 1877). However, at the time, Myxomycetes were regarded as fungi, including *P. brassicae* and other plasmodiophorids. Common with fungi is a chitinous cell wall of the resting spores (Muirhead & Pérez-López, 2022; Thornton et al., 1991), but distinctive features including cruciform nuclear division, biflagellate secondary zoospores, multinucleate plasmodia, uninucleate resting spores, and lack of filamentous growth make plasmodiophorids clearly divergent from fungi and oomycetes (Bulman et al., 2011; Neuhauser et al., 2014).

With the progress of DNA sequencing, it was shown that plasmodiophorids belong to the Phytomyxea (Endomyxa) in the eukaryotic Rhizaria group (Bi et al., 2019; Neuhauser et al., 2014; Sierra et al., 2015). The Phytomyxea consists of obligate biotrophic parasites of brown algae, oomycetes (Phagomyxids), and a diverse range of plant hosts (Plasmodiophorids) and the newly described Marinomyxa (Hittorf et al., 2020; Kolátková et al., 2021; Neuhauser et al., 2014). Along with the clubroot pathogen, the causal agent of powdery scab potato, *Spongospora subterranea*, is another well-known plant-pathogen member of Phytomyxea (Falloon et al., 2016).

3 | THE CLUBROOT PATHOGEN LIFE CYCLE

The clubroot pathogen is a biotrophic obligate plant parasite that requires the plant host to complete its life cycle (Kageyama & Asano, 2009). To this day, the clubroot pathogen has not been cultured in axenic media, despite multiple tries, although it has been possible to see different *P. brassicae* developmental stages when callus was generated from infected brassica roots (Ingram, 1969; Tu et al., 2019). Although the work with this pathogen is very challenging, in the last 10 years significant progress has been made clarifying several controversial points in the *P. brassicae* life cycle, that is, where primary and secondary infection takes place in the roots and if a sexual life stage exists (Auer & Ludwig-Müller, 2015; Liu, Qin, Zhou, et al., 2020).

The *P. brassicae* life cycle is divided into two main phases: (i) the primary infection and (ii) the secondary infection of the cortex tissue (Figure 2) (Kageyama & Asano, 2009). The primary infection was

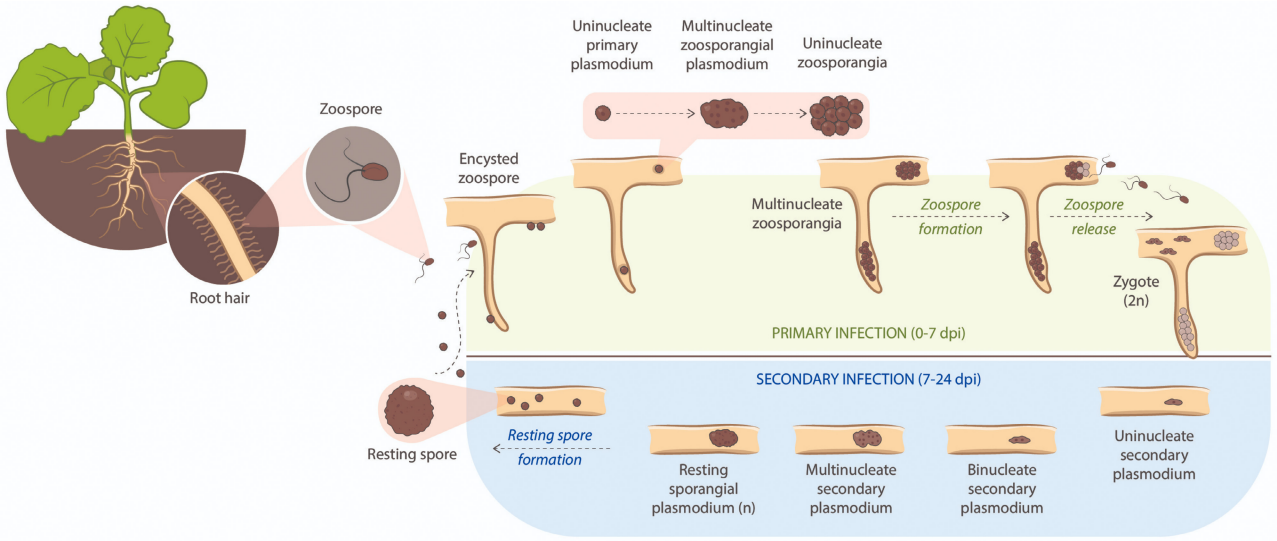

FIGURE 2 Representation of *Plasmodiophora brassicae* life cycle. Life cycle is typically divided into primary infection in host root hairs and epidermal cells (top panel in yellow) and secondary infection in the cortical tissues (bottom panel in blue). Primary infection starts with resting spores in soil that germinate to produce primary zoospores. Around 1 day postinoculation (dpi) the encysted primary zoospore punctures the host cell wall and an uninucleate primary plasmodium is generated in root hairs or epidermal cells. Next, from 1 to 3 dpi, a multinucleate zoosporangial plasmodium is produced as a result of mitotic divisions of the uninucleate primary plasmodium, followed by the formation of an uninucleate zoosporangial cluster from 3 to 4 dpi. From 4 to 7 dpi a multinucleate zoosporangium is formed that then yields uninucleate secondary zoospores within each multinucleate zoosporangium and secondary zoospores are released from each zoosporangium into the lumen of root hairs or epidermal cells, concluding with the conjugation of two haploid uninucleate secondary zoospores in the root epidermal cell resulting in the formation of a diploid uninucleate zygote. Secondary infection starts with the formation of a uninucleate secondary plasmodium at 8 dpi in the cortical cell. Next, a binucleate secondary plasmodium is formed at 10 dpi and a multinucleate secondary plasmodium is produced at approximately 15 dpi. Finally, a haploid multinucleate resting sporangial plasmodium is formed, and haploid uninucleate resting spores split from the haploid multinucleate resting sporangial plasmodium around 24 dpi. Model based on Liu, Qin, Cheng, et al. (2020)

considered to occur exclusively in the root hairs, but recently it has been shown that primary infection can also occur in epidermal cells in the root elongation zone, the zone where the gall initiates (Liu, Qin, Zhou, et al., 2020; Tu et al., 2019). After resting spores germinate, primary zoospores are released and primary infection is established in root hairs or epidermal cells passing from uninucleate primary plasmodium to a diploid uninucleate zygote (Figure 2) (Liu, Qin, Zhou, et al., 2020).

Around 7 days postinoculation, the secondary infection occurs, starting with the formation of uninucleate secondary plasmodium by the karyogamy of secondary zoospores (Liu, Qin, Zhou, et al., 2020). An important recent finding is that based on reducing the nuclear size from secondary plasmodia to resting spore, meiosis may occur to restore the haploid life stage of *P. brassicae* (Liu, Qin, Zhou, et al., 2020). While there is as yet no consensus regarding the presence of a sexual stage in *P. brassicae*, these findings suggest that such a stage may occur during the conjugation of secondary zoospores to the formation of resting spores (Figures 2 and 3a) (Liu, Qin, Zhou, et al., 2020).

Recent findings by Liu, Qin, Zhou, et al. (2020) indicate a highly asynchronous development of *P. brassicae*, with different stages consistently occurring together during infection. During early primary infection, a low abundance of uninucleate primary plasmodium and binucleate primary plasmodia can be found in root hairs and epidermal cells. With the progress of the primary infection, a combination of multinucleate primary plasmodia, zoosporangia, empty zoosporangia, and decreasing proportions of uninucleate and primary plasmodia can be found (Liu, Qin, Zhou, et al., 2020). In the early stages of the secondary infection, high proportions of uninucleate and binucleate secondary plasmodia are present in infected cortical cells, progressing to more multinucleate secondary and sporulating plasmodia and resting spores at later stages (Liu, Qin, Zhou, et al., 2020).

4 | CLUBROOT DISEASE

The first description of clubroot disease dates back to the fourth century AD, when Pallidus reported the development of spongy type roots of radish, turnip, and rape (Watson & Baker, 1969). There are credible reports of clubroot on cabbages in Spain from the 15th century, and an illustration by Fuchs in 1542 showing large spherical galls on *Brassica oleracea* roots might be the first illustration of clubroot symptoms (Woronin, 1934). After an epidemic outbreak in St Petersburg, Russia, the responsible pathogen *P. brassicae* was

FIGURE 3 Clubroot spores and disease symptoms. (a) Scanning electron micrographs of immature resting spores in clubroot of canola in xylem vessels. Scale bar = 5 μm. (b) Galls in *Brassica napus* roots. (c) Aboveground symptoms in *Arabidopsis thaliana*

identified and described by Woronin (1877). Nevertheless, even though the disease and the causal agent have been long known, clubroot has been a persistent problem for the cultivation of Brassicaceae, and it still has an enormous impact on the cultivation of brassica crops worldwide (Dixon, 2009; Ludwig-Müller, 2022).

The initiation of disease symptoms and time for infection depends on the host type, virulence of the pathotype, number of resting spores in the soil, as well as optimum environmental factors including soil temperature, humidity, and pH (Arvelius, 2021; Hwang, Strelkov, et al., 2012; Tso et al., 2021; Zamani-Noor et al., 2022). Severely infected roots by the clubroot pathogen characteristically become swollen, form galls, and induce hypocotyl deformation, which explains why the disease is called clubroot (Figure 3b) (Malinowski et al., 2019). Host clubbed roots provide a nutrient and food sink, translocated from the upper parts of the plant for *P. brassicae* growth and survival. Hence, the infected plant shows reduced growth compared to those noninfected with clubroot pathogen (Figure 3c). The aboveground organs ripen prematurely and seeds shrivel in severe infection (Botero et al., 2019). Moreover, the swollen roots stop nutrient and water uptake, resulting in the young leaves turning green to pale green and then yellow, followed by dehydration, and ultimately wilting and stunting of the whole plant (Figure 3c) (Howard et al., 2010).

During disease progression, it has been found that downstream cellular modifications are more conserved in roots and hypocotyl. These cellular changes in meristematic activity enhance the proliferation of cells accompanied by belowground parts of the plant (Malinowski et al., 2019). In *Arabidopsis thaliana* (hereafter Arabidopsis), it has been found that galls have increased cambial cellular division leading to cell hyperplasia, while cambial cell differentiation remains intact (Malinowski et al., 2019; Olszak et al., 2019). Interestingly, during early disease progression, the clubroot pathogen suppressed xylogenesis, a key component of xylem differentiation during normal plant growth (Olszak et al., 2019). Similarly, the phloem bundles are also affected by an imbalance of cell proliferation and differentiation in hypocotyl during *P. brassicae* infection (Slewinski et al., 2013). The pathogen targets the phloem lineage cells to proliferate, which are responsible for the translocation of sugar compounds, providing the perfect environment for *P. brassicae* propagation and maturation (Figure 3a) (Olszak et al., 2019; Slewinski et al., 2013).

Genomics and transcriptomics analysis documented that *P. brassicae* relies on carbohydrates, mainly sucrose, which is abundantly found at the infection site and translocates from source to sink as the disease progresses (Hwang, Strelkov, et al., 2012; Malinowski et al., 2019). It has been reported that photosynthesis and starch-related genes in the host are also suppressed on disease progression. In response, the infected plant limits the production of carbohydrates at the source site, but the clubroot pathogen increases carbohydrate metabolism at the infection site as a counter-effect (Irani et al., 2018). Genomic data revealed that the clubroot pathogen does not have an invertase gene for sink carbohydrates metabolism. Instead, new findings showed that the pathogen acquires carbohydrates directly from the host through transporter proteins or re-orientation of carbohydrates via trehalose (Olszak et al., 2019; Rolfe et al., 2016; Schwelm et al., 2015, 2016).

5 | THE CLUBROOT HOST RANGE

Theoretically, *P. brassicae* can only infect members of the Brassicaceae family. However, several studies have identified that the clubroot pathogen can infect other plant species outside the Brassicaceae family, serving as a source of inoculum in the field (Dixon, 2009). In recent years, the use of cover crops has been gaining importance worldwide, including among important canola producers like Canada and European countries, as their application has numerous advantages, such as containing reduction in soil erosion, retention of nutrients, broadening of soil organic matter, management of water runoff, and increase in water infiltration (Blanco-Canqui et al., 2012; Brust et al., 2014). Some studies indicated that germination of *P. brassicae* resting spores might be stimulated by nonhost plant species such as rye, leek, or perennial ryegrass. However, these germinated spores cannot infect these plants (Hwang et al., 2015). Even without a secondary infection and the development of clubs in the roots, the spore load in the soil can rise significantly in the consecutive growing seasons through primary infection alone (Dixon, 2014; Liu, Qin, Zhou, et al., 2020; Zamani-Noor & Rodemann, 2018; Zamani-Noor et al., 2021).

In a recent study, the effect of *P. brassicae* virulence on clubroot disease development and resting spores reproduction was evaluated in different weeds, cash, and cover crops from 19 plant families (Zamani-Noor et al., 2022). Plants were inoculated with two isolates of *P. brassicae* that differed in aggressivity: *P. brassicae*-P1 (+), which is virulent on the clubroot-resistant canola cultivar Mendel, and *P. brassicae*-P1, which is avirulent on this cultivar (Zamani-Noor, 2017). The expected clubroot symptoms were identified mainly in plants in the Brassicaceae family, with a differential response depending on the virulence of the isolate used during the infection (Figure S1).

Among all brassicaceous species tested, hill mustard, creeping watercress, and garden radish were thoroughly resistant against both isolates. In contrast, flat-seed false flax, shepherd's purse, wallflower cabbage, flix weed, hairy rocket, yellow ball mustard, white mustard, charlock, Jim hill mustard, false London rocket, and field pennycress were significantly susceptible to both clubroot pathogen isolates. However, hare's-ear cabbage, slim-leaf wall rocket, hoary mustard, annual candytuft, and field pepper weed were entirely or partially resistant to *P. brassicae*-P1 but clearly susceptible to P1 (+) (Figure S1) (Zamani-Noor et al., 2022). These results suggest that the basis for the resistance in the species mentioned above could represent what happens in some commercial crop species. Additionally, these species might contribute to an increase in resting spores of virulent pathotypes, and to fully identify if a plant can be a host or not of the clubroot pathogen it would be necessary to use highly virulent isolates.

6 | CLUBROOT DETECTION

Efficient disease management starts with a reliable and sensitive diagnostic method to stop the spread of the pathogen and its economic losses. Although this area has also been very challenging, in the last decade several molecular methods have been developed to detect *P. brassicae* resting spores in infected fields, if possible before typical symptoms can be observed (Gossen et al., 2019; Tso et al., 2021, 2022). In addition, several PCR-based assays have been developed in the last 10 years targeting diverse genes aiming to detect the pathogen in infected fields, quantify the infection load, or differentiate *P. brassicae* pathotypes with different virulence profiles (Czubatka-Bieńkowska et al., 2020; Gossen et al., 2019; Tso et al., 2021, 2022).

The nuclear ribosomal DNA (rDNA), comprising small subunits (SSUs), a large subunit (LSU), internal transcribed spacers (ITS1 and ITS2), and a 5.8S subunit, has been extensively used as a marker to identify *P. brassicae* and to differentiate geographical isolates of the pathogen (Li et al., 2013; Padrón-Rodríguez et al., 2022; Schwelm et al., 2016; Schwelm & Neuhauser, 2017; Zhou et al., 2018). There is evidence that shows that single nucleotide polymorphisms, insertions, and deletions in these regions can differentiate *P. brassicae* isolates based on geographic origin (Schwelm & Neuhauser, 2017) but also based on their virulence, for example the case of the highly virulent pathotype 5, now Pb5X, identified in Canada (Table 1) (Zhou et al., 2018). These markers have also been extensively used to develop quantitative PCR assays to study the distribution of the clubroot pathogen in fields and other epidemiological aspects of the disease (Table 1) (Cao et al., 2014; Czubatka-Bieńkowska et al., 2020; Deora et al., 2015; Tso et al., 2022; Wallenhammar et al., 2012).

With the discovery of new and diverse pathotypes, the challenge has been to identify areas of the genome useful to differentiate virulent from avirulent pathotypes in combination with more resolutive methods (Tso et al., 2022; Zheng et al., 2018). An example of this is the use of *P. brassicae cpn60* universal target (*cpn60*UT) to develop digital droplet PCR (ddPCR) and loop-mediated isothermal DNA amplification (LAMP), which is proving to be a suitable market to study the distribution of the pathogen in infected fields and to differentiate pathotypes (Table 1) (Gossen et al., 2019). Another method extensively used to differentiate clubroot pathotypes is RNase H-dependent PCR (rhPCR) (Tso et al., 2022; Yang et al., 2018), which amplifies rDNA (Yang et al., 2018) or fragments of the genome unique for different pathotypes identified through comparative genomics (Table 1) (Tso et al., 2022).

Visible/near-infrared hyperspectral (HSI) imaging with convolutional neural networks (CNN) is another technique used to detect clubroot by estimating the plant physiological abnormalities induced by the clubroot pathogen (Feng et al., 2022). However, this technique has considerable limitations because it only detects the disease after successful infection has been established and the plant shows visible symptoms, when the resting spores are already spread through the field (Table 1).

7 | PATHOTYPING THE CLUBROOT PATHOGEN

From very early on in clubroot research a differential physiological response of brassicas to the clubroot pathogen was observed, which was used to differentiate *P. brassicae* isolates as races (Ayers, 1957; Buczacki et al., 1975; Williams, 1966). Today, instead of races, the term pathotypes is used to distinguish between *P. brassicae* isolates with differential virulence profiles in brassica species because the genetics of the interaction are not well enough defined to apply the concept of races to clubroot (Somé et al., 1996; Strelkov et al., 2018), although technically speaking we should be able to find different races of *P. brassicae* into those classified as a certain pathotype, as previously described for other pathogens (Bourras et al., 2019).

Pathotyping is very important because without knowledge of the existing pathotypes in the field and their virulence, it can be difficult to breed for appropriate resistance in host cultivars and for growers to select the best variety or cultivar. Efforts have therefore been made worldwide to develop an accurate and reproducible pathotyping system of *P. brassicae* (Pang et al., 2020; Somé et al., 1996; Strelkov et al., 2018; Williams, 1966). Nevertheless, the task remains

TABLE 1 Detection methods developed to study *Plasmodiophora brassicae* in the last 10 years

Method	Applications	References
PCR	Detection of *P. brassicae*, differentiation of *P. brassicae* pathotypes based on virulence or geographic distribution	Li et al. (2013); Schwelm and Neuhauser (2017); Zhou et al. (2018)
qPCR	Study of *P. brassicae* distribution and quantification of spore number in the field	Al-Daoud et al. (2017); Cao et al. (2014); Czubatka-Bieńkowska et al. (2020); Deora et al. (2015); Jonsson et al. (2016); Wallenhammar et al. (2012)
ddPCR	Quantification of *P. brassicae* and study of the pathogen dynamics in the field	Gossen et al. (2019); Wen et al. (2020)
rhPCR	Study of *P. brassicae* population polymorphisms and differentiation of clubroot pathotypes with differential virulence profiles	Tso et al. (2022); Yang et al. (2018)
LAMP	Fast and sensitive detection of *P. brassicae*	Gossen et al. (2019); Yang et al. (2022)
HSI + CNN	Detection of clubroot disease without destroying the plants infected	Feng et al. (2022)

CNN, convolutional neural networks; ddPCR, droplet digital PCR; HSI, hyperspectral imaging; LAMP, loop-mediated isothermal amplification; qPCR, quantitative PCR; rhPCR, RNase H-dependent PCR.

challenging because the majority of the pathotyping studies conducted to this day do not include commercial brassica varieties and the results are subject to the genetic variability linked to the fact that most of the crops in this family are polyploid and none of the phenotyping systems have been developed using clones that could ensure reproducibility (Pang et al., 2020; Somé et al., 1996; Strelkov et al., 2018; Tso et al., 2021; Williams, 1966).

For many years three pathotyping systems have been widely used: Williams clubroot differential (Williams, 1966), European clubroot differential (ECD) (Buczacki et al., 1975), and Somé clubroot differential (Somé et al., 1996). However, during the early 2000s, clubroot-resistant (CR) cultivars began to appear in the market, requiring changes to the way we used to differentiate *P. brassicae* isolates (Hwang, Strelkov, et al., 2012). For instance, the first CR canola (45H29) was introduced in Canada in 2009. Since then, many CR varieties have been generated, introgressing CR genes from other crops like rutabaga or Chinese cabbage (Liu et al., 2018). Currently, there are over 20 registered CR canola varieties in Canada. However, due to this high selection pressure, the resistance in most commercial cultivars has been overcome by the emergence of new virulent pathotypes, leading to the introduction of a new clubroot differential system known as the Canadian Clubroot Differential (CCD) (Strelkov et al., 2018). More than 35 different clubroot pathotypes have been identified in Canada using the CCD, using 13 hosts, one of them being a CR *Brassica napus* for the first time (Figure S2) (Strelkov et al., 2018). The most recent differential system is the Sinitic clubroot differential (SCD), which theoretically can detect more than 250 pathotypes from brassica crops in Korea and China (Pang et al., 2020).

Although many different pathotyping systems are available, unfortunately their applicability is complicated, and there are some drawbacks associated with these approaches. One is the feasibility and labour-intensive nature of the experiments, which makes it time-consuming as the whole process from seedling to the evaluation of galls takes from 7 to 8 weeks, and this process must be repeated for statistical validation (Pang et al., 2020; Somé et al., 1996; Strelkov et al., 2018; Williams, 1966). Furthermore, the final evaluation of the galls for disease indexing takes place based on a visual estimation method that can be error-prone and thus impact the reproducibility and reliability of the results obtained (Siemens et al., 2002). This has motivated the development of molecular pathotyping aiming to connect phenotype with genotype with some degree of success (Tso et al., 2022; Zhou et al., 2018). However, there is still no phenotyping method to indicate to a grower, based on a screening of *P. brassicae* phenotypes present in their field, which variety or cultivar will be the best to grow to avoid the economic losses linked to clubroot disease.

8 | THE CLUBROOT PATHOGEN GENOME

P. brassicae remains impossible to grow in axenic culture due to intracellular growth in the host cells and obligate biotrophy, preventing many experimental procedures used in other systems. Thus, the first genome of the pathogen was only published in 2015 (Schwelm et al., 2015), which was relatively late compared to other plant-pathogenic organisms with similar agricultural and economic impacts. The first draft genome was obtained for the European single SSI e3 isolated from a stubble turnip, estimated by electrophoretic karyotype to have 16 chromosomal bands and a size of 20.3 Mb (Graf et al., 2001, 2004). The genome draft was generated using 454 DNA sequencing and Illumina short sequencing reads technologies and assembled into 165 contigs and a total size of 24 Mb (Schwelm et al., 2015). Building on that first e3 assembly and using PacBio long sequencing technology, a nearly complete assembled genome version of the e3 genome was achieved four years later (Stjelja et al., 2019). This genome has a total size of 25.1 Mb and includes 19 nuclear contigs, of which 13 are assembled telomere to telomere, and a circular mitochondrial genome of 114.6 kb. The high quality of the e3 genome will benefit future genome assemblies of *P. brassicae* isolates and comparative genomics of plasmodiophorids.

Subsequent to the first release of the e3 genome, draft genomes of several canola isolates from Canada (Rolfe et al., 2016), China (Bi et al., 2019), and Europe (isolate eH; Daval et al., 2019), which derived from the same club as the e3 isolate, were generated. As some data remained unavailable to the scientific community for at least four years, the progress of the comparative genomics of *P. brassicae* isolates has been very slow. Recently, a single-nucleotide polymorphism analysis of *P. brassicae* genomic sequences of 34 fields and nine SSIs from Canada, the United States, and China compared to the e3 genome revealed at least five different clusters that do not correlate with the pathotypes (Sedaghatkish et al., 2019). There are now 50 draft genomes deposited publicly in the NCBI database (https://www.ncbi.nlm.nih.gov/genome/browse/#!/eukaryotes/38756/). There are reports of other genome sequences from Asian and Canadian *P. brassicae* isolates, but the data are not publicly available (Tso et al., 2022).

Assemblies in the NCBI range between 24.05 and 25.25 Mb. The relatively small genome size is due to a low instance of repeated sequences (up to 11%) and a reduction of intergenic elements in the genome (Rolfe et al., 2016; Schwelm et al., 2015; Stjelja et al., 2019). The number of protein-encoding genes is reported to range from 9231 to 12,811 (Daval et al., 2019; Rolfe et al., 2016; Schwelm et al., 2015; Stjelja et al., 2019), but gene models are only published for the version e3 genomes (9730, Schwelm et al., 2015; and 9231, Stjelja et al., 2019). Current data have already facilitated the generation of genome drafts for *S. subterranea* and *Polymyxa betae* and subsequent genomic analyses (Ciaghi et al., 2018; Decroës et al., 2022). Orthologue clustering of the protein models revealed 2296 proteins exclusive for *P. brassicae* compared to *S. subterranea* and *P. betae*, and a plasmodiophorid core proteome formed by 4885 orthologues (Decroës et al., 2019).

The currently available genome data reveal some features of *P. brassicae* also seen in other biotrophic plant pathogens, such as incomplete metabolic pathways that highlight the dependency of the pathogen on the host. Based on the annotated genomes *P. brassicae* appears to lack the ability to synthesize fatty acids

de novo, and transcriptional data suggest that fatty acid synthesis occurs in the plasmodia and degradation in germinated spores (Schwelm et al., 2015). High amounts of lipid bodies/organelles/structures in the resting spores (Bi et al., 2016) might act as a lipid reservoir for the clubroot pathogen until it can refuel after a host infection. However, *P. betae* spores are also filled with lipids, but fatty acid synthase-related genes are present in the genome (Decroës et al., 2019).

Approximately half of the predicted *P. brassicae* proteins show no similarities to proteins from other species, lack known functional domains, or have only a predicted unknown or general function (Rolfe et al., 2016; Schwelm et al., 2015; Stjelja et al., 2019). Additionally, genomic data for Rhizaria, the eukaryotic group to which plasmodiophorids belong, is only marginal compared to other eukaryotic groups (Burki & Keeling, 2014; Sibbald & Archibald, 2017), therefore functional analyses will remain important in elucidating *P. brassicae* biology. This is especially true for effector candidates as they play a significant role in the pathogenicity and virulence of plant pathogens.

9 | CLUBROOT PATHOGEN EFFECTORS

After millions of years of coevolution, plants have developed a complex innate immune system to protect them against all types of pathogens (Sacristán et al., 2021; Zhou & Zhang, 2020). During the colonization process, the pathogens must first avoid the immune perception and/or suppress immune responses and, at the same time, derive the nutrients needed to ensure their survival (Dodds & Rathjen, 2010). To achieve this goal, some pathogens, such as *P. brassicae*, have developed a range of secreted molecules called effectors to promote plant colonization (Pérez-López et al., 2020; Sacristán et al., 2021; Snelders et al., 2018). Originally, pathogen effectors were defined as small, cysteine-rich proteins with a role in the manipulation of plant immune responses (Pérez-López et al., 2018). However, recent work has demonstrated pathogen effectors also acting in pathogen self-defence, showing nutrient derivation towards infected tissues, or promoting cytotoxicity, among many other functions (Lo Presti et al., 2015; Sacristán et al., 2021; Snelders et al., 2018). Likewise, some other secreted molecules, such as small RNA or secondary metabolites, have been described as exerting typical effector functions, forcing a revision of the effector definition (Seto et al., 2021; Wang & Dean, 2020).

Several factors have made the discovery and characterization of effectors nontrivial, for example the lack of common features or sequence homologies. Consequently, in most of the species, as in the clubroot pathogen, the complete set of effectors is not still well defined, and their precise function, as well as their targets in the host, remain unknown (Pérez-López et al., 2018; Sacristán et al., 2021). With the rise of *P. brassicae* genomic data and protein models, the research into potential effector candidates has been boosted. However, the first effector candidate was identified and characterized prior to the availability of whole genome data (Ludwig-Müller et al., 2015). This was PbBSMT, a protein with homologies to plant SABATH-type methyltransferases (Ludwig-Müller et al., 2015), whose gene sequence was obtained from a dataset of differentially expressed *P. brassicae* genes (Bulman et al., 2006, 2011). In vitro, PbBMST converts the defence compound salicylic acid (SA) to its methyl ester (Me-SA) when expressed in *Escherichia coli* (Ludwig-Müller et al., 2015). Subsequently, PbBMST has been shown to be one of the highest expressed *P. brassicae* genes in infected root tissues (Ciaghi et al., 2018; Rolfe et al., 2016; Schwelm et al., 2015) and has been shown to be associated with the plasmodial stage of the pathogen (Badstöber et al., 2017). Arabidopsis plants overexpressing PbBSMT exhibited a higher Me-SA versus SA content and were more susceptible to other pathogens (Bulman et al., 2019, Djavaheri et al., 2018). In the roots of transgenic Arabidopsis with a constitutive SA response, the PbBSMT was down-regulated and plants showed tolerance to clubroot infection (Mencia et al., 2022). This SA-manipulating type of effector has so far only been detected in *P. brassicae* and not in other plant-pathogenic organisms and is also absent in the genome drafts of the plasmodiophorids *S. subterranea* and *P. betae* (Ciaghi et al., 2018; Decroës et al., 2022). Thanks to the availability of genome sequences and transcriptome data, more effector protein candidates have been identified (Chen et al., 2019; Daval et al., 2020; Galindo-González et al., 2021; Holtz et al., 2018; Hossain et al., 2021; Pérez-López et al., 2020; Rolfe et al., 2016; Schwelm et al., 2015).

Among predicted effectors, proteins with ankyrin repeats and chitin-binding domains are commonly found, as well as putative kinases and small cysteine-rich proteins (Galindo-González et al., 2021; Hossain et al., 2021; Pérez-López et al., 2020; Schwelm & Ludwig-Müller, 2021). Several effectors have been characterized out of these genomic and transcriptomic studies, such as the cysteine protease inhibitor SSPbP53, which is able to interact with the xylogenesis-related papain-like cysteine protease XCP1 and plays a role in allowing clubroot disease to progress (Pérez-López et al., 2021). Other examples are a set of effector candidates able to suppress or induce cell death in hosts and nonhosts plants (Chen et al., 2019; Hossain et al., 2021), and PBZF1, an RxLR effector able to interact with Arabidopsis SnRK1.1 kinase to promote *P. brassicae* virulence (Chen et al., 2021). A recent study focused on the identification of putative effectors with chitin-binding domains identified effectors PbChiB4 and PbChiB2, bearing two carbohydrate-binding module family 18 (CBM18), and able to bind to chitin and resting spores, and suppress chitin-triggered immunity in canola (Table 2) (Muirhead & Pérez-López, 2022).

Several candidate effector lists have been generated using different criteria, but one question remains: which candidate effectors should we continue characterizing first? After analysing all the different subsets, we have identified those candidate effectors that appeared in more than one list (Table 1). However, more than 40% of the candidate effectors in this combined list are hypothetical proteins with unknown functions or homologous, calling for the use of new tools like AlphaFold or ColabFold to identify if those *P. brassicae* proteins are sequence-unrelated but structurally similar (SUSS) to other

TABLE 2 List of *Plasmodiophora brassicae* effector candidates identified in more than one dataset reported to date

Gene ID	Effector candidate/functional domains	Alphafold predicted structure[a]	Reference
PBRA_001987	PBZF1/MYND finger	f.	Chen et al. (2019); Galindo-González et al. (2021)
PLBR_LOCUS9261	PbBSMT/methyltransferase	a.	Galindo-González et al. (2021); Pérez-López et al. (2020); Schwelm et al. (2015)
PBRA_000619	n.d.	k.	Chen et al. (2019); Hossain et al. (2021)
PBRA_001067	Serine/threonine protein kinase	j.	Hossain et al. (2021); Pérez-López et al. (2020)
PBRA_001204	ANK	z.	Chen et al. (2019); Galindo-González et al. (2021); Schwelm et al. (2015)
PBRA_001454	n.d.	q.	Chen et al. (2019); Schwelm et al. (2015)
PBRA_001722	ANK	w.	Hossain et al. (2021); Pérez-López et al. (2020)
PBRA_001856	HP	o.	Chen et al. (2019); Pérez-López et al. (2020)
PBRA_002100	SMC_prok_A	u.	Chen et al. (2019); Pérez-López et al. (2020)
PBRA_002462	n.d.	p.	Chen et al. (2019); Schwelm et al. (2015)
PBRA_003268	n.d.	i.	Galindo-González et al. (2021); Schwelm et al. (2015)
PBRA_003620	Cons. HP	y.	Chen et al. (2019); Pérez-López et al. (2020)
PBRA_004344	n.d.	c.	Chen et al. (2019); Hossain et al. (2021)
PBRA_004763	PRK14510	t.	Chen et al. (2019); Hossain et al. (2021)
PBRA_005440	HP	x.	Chen et al. (2019); Galindo-González et al. (2021); Pérez-López et al. (2020)
PBRA_005499	Cons. HP	m.	Chen et al. (2019); Pérez-López et al. (2020); Schwelm et al. (2015)
PBRA_005609	n.d.	v.	Galindo-González et al. (2021); Hossain et al. (2021)
PBRA_006323	n.d.	e.	Galindo-González et al. (2021); Hossain et al. (2021)
PBRA_006649	Ank_4	l.	Chen et al. (2019); Galindo-González et al. (2021); Pérez-López et al. (2020)
PBRA_006655	ANK	d.	Chen et al. (2019); Galindo-González et al. (2021); Pérez-López et al. (2020)
PBRA_007344	Cons. HP	c.	Hossain et al. (2021); Pérez-López et al. (2020)
PBRA_007868	Lactamase_B	b.	Chen et al. (2019); Hossain et al. (2021)
PBRA_008207	SSPbP53/cysteine protease inhibitor	g.	Hossain et al. (2021); Pérez-López et al. (2020)
PBRA_008279	Zinc finger C2H2-type domain	h.	Pérez-López et al. (2020)
PBRA_001907	PbChiB2/carbohydrate-binding module family 18	s.	Muirhead and Pérez-López (2022); Schwelm et al. (2015)
PBRA_002958	PbChiB4/carbohydrate-binding module family 18	g.	Muirhead and Pérez-López (2022); Schwelm et al. (2015)

[a]The letters indicate the position of this effector in Figure 4. n.d., nondetermined; HP, hypothetical protein.

members of the Rhizaria supergroup, as has been recently done with other pathosystems (Seong & Krasileva, 2021, 2022). With the development and continuous updates of AlphaFold, we are now closer to better understanding the *P. brassicae* secretome and effectorome (Jumper et al., 2021). Indeed, the latest structure prediction of the protein universe includes the more than 9000 proteins encoded by the *P. brassicae* genome (https://alphafold.ebi.ac.uk/search/text/plasmodiophora%20brassicae), allowing us to obtain the structure of the 25 candidate effectors in the combined list created for this review (Figure 4). For a more detailed information about the host response to clubroot infection, we refer the reader to several recent excellent reviews (Ludwig-Müller, 2022; Shaw et al., 2022).

10 | CLUBROOT MANAGEMENT

While we learn about the molecular strategies used by the clubroot pathogen to cause millions of dollars in economic loses, agronomic-based solutions keep being developed and implemented. Biological control, chemical control, and the development of genetic resistance are some of the strategies that can be developed in parallel to the study of *P. brassicae* genome and effectoromics to enrich each other. Other measures are more easily applied than others, but should be adapted to each country or carefully tailored by each grower trying to stop the spread of the disease in their region.

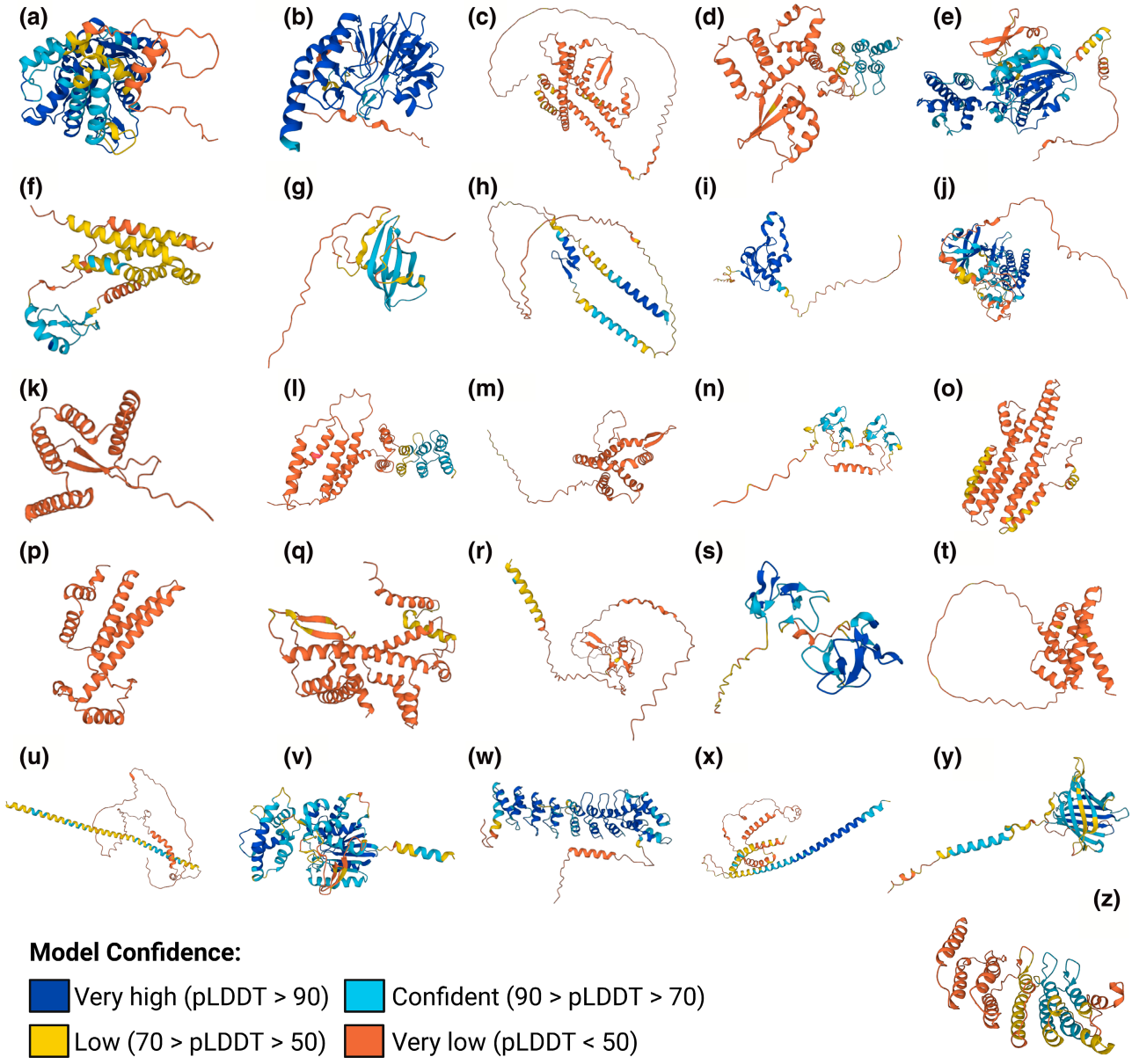

FIGURE 4 Structural prediction of *Plasmodiophora brassicae* effectors and candidate effectors identified in more than one study and described in Table 2. The colour of the structures is linked to the prediction score from AlphaFold

10.1 | Field hygiene, sanitation, control of weeds, and volunteer cruciferous crops

Sanitation is a well-known part of any clubroot management scheme and may help to slow disease spread of the clubroot pathogen in some areas, such as in Saskatchewan, Canada (Government of Saskatchewan, 2021; Hwang et al., 2014). Sanitation of field equipment and machinery that could have encountered clubroot-infested soil particles is a practice that is intended to slow the spread and development of clubroot disease. Moreover, adequate weeds and volunteer cruciferous management that may provide alternative hosts for the clubroot pathogen are also important for clubroot outcome (Hennig et al., 2022; Zamani-Noor & Rodemann, 2018).

10.2 | Crop rotation

Several studies have evaluated the impact of crop rotation in reducing *P. brassicae* resting spores in fields (Ernst et al., 2019; Hwang et al., 2019; Peng et al., 2015). A standard recommendation is to have more than 2 years of rotations with other nonhost crops, which can improve plant height and seed yield, and reduce clubroot severity and resting spores load in the fields (Hwang et al., 2019; Peng et al., 2015). These recommendations have evolved with the introduction of new CR cultivars, as a high reduction of clubroot propagule in the soil when different CR cultivars are used has been found, reducing the chances of experiencing a breakdown of the resistance and the proliferation of highly virulent populations of the clubroot pathogen (Hwang et al., 2019).

10.3 | Seeding date

Optimal environmental conditions are essential for *P. brassicae* resting spore germination and clubroot disease progress. Previous studies showed that high soil humidity and temperatures from 22 to 25°C are favourable for clubroot disease development (Feng et al., 2010; Gossen et al., 2012; Hwang et al., 2011; Sharma et al., 2011). Additionally, it has been demonstrated that pathogen growth is inhibited by cold soil temperatures (Hwang et al., 2011). Furthermore, it has been shown that younger plants are more susceptible to clubroot infection, and the infection that does happen is more likely to affect yield (Hwang et al., 2011); therefore, scheduling the sowing date properly can help prevent clubroot infection and its economic losses.

10.4 | Soil amendments

An acidic soil environment is frequently favourable for clubroot development, therefore lime-based products have been the primary strategy to change soil pH to 7.0 or higher and decrease the incidence of clubroot disease (Botero et al., 2019; Fox et al., 2022; Hennig et al., 2022; McGrann et al., 2016). Several studies have assessed the effect of soil amendment with boron, calcium cyanamide, and calcium carbonate alone or in various combinations applied before sowing for the potential to control clubroot, all with variable degrees of success in experimental fields (Botero et al., 2019; Deora et al., 2013; Fox et al., 2022; Gossen et al., 2014; Hennig et al., 2022; McGrann et al., 2016). However, the high cost of these methods makes their extensive application difficult, as shown in Latin American countries (Botero et al., 2019).

10.5 | Bait crops

Resting spores can survive for several years in the soil without the host plant. However, they must quickly infect a host following germination to ensure survival; therefore, inducing germination of resting spores in the absence of host plants has been recommended as a possible tool in managing clubroot disease (Ahmed et al., 2011). Some plant species have been proved to induce germination of *P. brassicae* resting spores without becoming infected and have been identified as bait crops (Ahmed et al., 2011; Zamani-Noor et al., 2022). Further research has shown the potential use of black grass, phacelia, field poppy, and field pea as bait crops to reduce the number of resting spores in clubroot-infected fields (Zamani-Noor et al., 2022).

10.6 | Fungicide application

The use of fungicides has been part of clubroot management strategies since the 1980s (Doyle & Clancy, 1987). Several synthetic fungicides, such as fluazinam, pentachloronitrobenzene, metalaxyl-mancozeb, azoxystrobin, difenoconazole, and carbendazim, have been tested against the clubroot pathogen. However, no consensus on these effects has been reached due to variable levels of control depending on the crop, geographical location, and application strategies (Hwang, Cao, et al., 2012; Liao et al., 2022; Peng et al., 2014). The discrete efficiency controlling clubroot, the high cost for growers, along with international strategies to reduce the inputs of synthetic pesticides in agriculture are reasons to keep exploring alternatives such as biological control and genetic resistance.

10.7 | Biological control

Biological control has caught the attention of clubroot researchers for its potential to be a successful and environmentally friendly tool to manage the disease. Among the several microorganisms with well-known biocontrol properties, *Trichoderma* spp. and *Bacillus* spp. have been extensively used to control clubroot disease in Asia, North America, and Latin America (Botero-Ramírez et al., 2015; Peng et al., 2014; Santos et al., 2017; Yu et al., 2015; Zhao et al., 2022; Zhu et al., 2020). For many years growers have been using *Trichoderma* and *Bacillus* strains without knowing how these microorganisms control clubroot but recently that has started to change. After analysing the genome of *Bacillus velezensis* F85 and *Bacillus amyloliquefaciens* T113, two strains highly effective in controlling clubroot disease, it was found that they harbour the genes responsible for antibiotic biosynthesis and antimicrobial peptide production (Zhu et al., 2020). For *Trichoderma* spp., the mechanism could be very different, and might imply the modulation of the rhizosphere microbial community of the inoculated plant and the recruitment of bacteria with biocontrol properties, such as *Bacillus* spp. (Li et al., 2020).

Another group of microorganisms that is being explored for biological control of clubroot is endophytes. The best example is the fungal endophyte *Acremonium alternatum*, which is not able to reduce clubroot symptoms in the roots but is successful in keeping the aboveground part of the plant healthier without compromising yield in canola, Chinese cabbage, and Arabidopsis (Auer & Ludwig-Müller, 2015; Doan et al., 2010; Jäschke et al., 2010). Although the mechanisms behind the phenotypic response observed in brassicas after treatment with *A. alternatum* are unknown, there is evidence pointing to an auxin-mediated response, probably triggered by immunity (Auer, 2020).

11 | CLUBROOT RESISTANCE

Plants rely on a two-layered innate immunity system to withstand various pathogen attacks, known as pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). However, recent discoveries have shown that the line between the layers is blurred (Ngou et al., 2021). Understanding the plant immunity system is crucial for protecting brassica oilseed and vegetable crops from clubroot disease. In the first layer of protection, plants use cell surface-localized

pattern recognition receptors (PRRs) to detect conversed pathogen-/microbe-associated molecular patterns (PAMPs/MAMPs), such as bacterial flagellin and fungal chitin, to activate PTI (Ngou et al., 2022). Plant PRRs are either receptor kinases or receptor-like kinases (RLKs), and ligand recognition by PRRs allows the plant to resist most nonadapted pathogens and provide basal immunity to adapted pathogens during early infection (Boutrot & Zipfel, 2017; Ngou et al., 2022). However, many pathogens can deliver virulence effector molecules to avoid or suppress PTI (Ngou et al., 2022; Seto et al., 2021). ETI is mediated by intercellular nucleotide-binding (NB) site leucine-rich-repeat (LRR) receptors (NLRs), which play an essential role in plant innate immunity (van Wersch et al., 2020). Activation of ETI triggers stronger resistance such as a hypersensitive response at the infection site, biosynthesis of pathogen-related phytohormones, and transcriptional reprogramming (Guo et al., 2020). NLR proteins in the plant can be classified into two major types based on their N-terminal domains: Toll/Interleukin-1 receptor/Resistance (TIR) protein NLRs (TNLs) and coiled-coil (CC) domain NLRs (CNLs) (van Wersch et al., 2020). Generally, the LRR domain acts as effector perception and the NB ATPase (NB-ARC) domain plays a central role in regulating activate or inactivate states of NLR by binding ATP or ADP, respectively (Monteiro & Nishimura, 2020). Oligomerization of the TIR or CC domain is required for interactions between resistance proteins and effectors, and the activation of defence signalling (Guo et al., 2020).

The NLR is the largest group of R proteins in plants. It is reported that there are 641 NLR genes in *B. napus*, 249 in *B. rapa*, and 443 in *B. oleracea* (Alamery et al., 2018). Genetic analysis and fine mapping studies have identified major loci, quantitative trait loci (QTLs) and genes from different *Brassica* species involved in *P. brassicae* resistance (Hasan, Shaikh, et al., 2021; Lv et al., 2020). Due to the lack of CR genes in *B. napus* (canola, genome AACC) germplasm, the canola progenitor *B. rapa* (Chinese cabbage, genome AA) is considered as a major source in CR breeding (Huang et al., 2017; Yang et al., 2022). Several candidate CR genes have been identified to encode NLR proteins in *B. rapa* (Hatakeyama et al., 2017, 2022; Huang et al., 2017; Matsumoto et al., 2012; Ueno et al., 2012; Wang et al., 2022; Yang et al., 2022; Zhang et al., 2021). *CRa* and *Crr1a* were successfully isolated and characterized from Chinese cabbage lines of *B. rapa*, and both confer resistance to clubroot and encode TNLs (Hatakeyama et al., 2013; Ueno et al., 2012). For instance, overexpression of $Crr1a^{G004}$ in susceptible *B. rapa* increased resistance to *P. brassicae* isolate Ano-01 (Hatakeyama et al., 2013).

Recently, Yang et al. (2022) sequenced the genome of *B. rapa* (ECD04) and identified 62 candidate CR genes, of which *CRA3.7.1* and *CRA8.2.4* were identified as NLRs and functionally validated by transforming susceptible *B. napus*. Interestingly, phylogenetic analysis indicated that the loss of CR function in susceptible *Brassica* species was affected by transposable element insertion after whole-genome triplication (Yang et al., 2022), suggesting the importance of comparison and evolutionary analysis of NLR genes for brassica CR breeding.

Another *B. rapa* CR locus recently fine mapped is the locus *CRA8.1*. It was found that this contains two different loci *CRA8.1a* and *CRA8.1b*, and those three genes could be responsible for the clubroot resistance, the TIR-NBS-LRRs *BraA08g039211E* and *BraA08g039212E*, and the receptor-like protein (RLP) *BraA08g039193E* (Wang et al., 2022). This last study is particularly important because it is the first time that an RLP has been suspected to provide clubroot resistance in brassicas. However, it should not be surprising considering that a recent study searching for resistance gene analogs (RGAs) in all available genome annotations from the family Brassicaceae identified 21,691 RLKs, 8588 NLRs, and 3786 RLPs, highlighting the chances to find CR gene members in any of these gene families (Tirnaz et al., 2020).

Gall formation during *P. brassicae* infection alters the host cell wall metabolism (Malinowski et al., 2012). The plant cell wall forms a dynamic physical barrier to protect cells during the infection of *P. brassicae* (Badstöber et al., 2017, 2020). The polysaccharides cellulose, hemicellulose, lignin, pectin, and structural proteins are the major components. Several studies have indicated the crucial role of lignin and pectin biosynthesis in inhibiting gall formation in CR plants (Irani et al., 2018; Lahlali et al., 2017; Miedes et al., 2014; Zhang et al., 2016). Furthermore, it has been reported that some cell wall components in CR canola roots, such as lignin and pectin, were significantly higher than in susceptible roots during *P. brassicae* infection, which suggests potential biomarkers for canola CR line selection in the future (Lahlali et al., 2017).

In addition, phytohormones play a critical role in clubroot resistance. It is well known that auxins and cytokinin biosynthesis are essential for root gall development (Hasan, Megha, et al., 2021; Ludwig-Müller et al., 1999; Siemens et al., 2006). In contrast, jasmonic acid (JA) and salicylic acid (SA) signalling pathways also contribute to clubroot resistance. The *cpr5-2* mutant, with constitutive induction of SA, is more resistant to clubroot than the wild type, and the mutant *jar1*, which is JA signalling-deficient, is more susceptible than the wild type in Arabidopsis (Lemarié et al., 2015). Moreover, it is reported that exogenous application of SA can reduce clubroot disease severity in *B. oleracea* and *B. campestris* (Lovelock et al., 2013; Xi et al., 2021).

It is well known that plant immunity is affected by growing temperatures (Kim et al., 2022; Qiu et al., 2022). Both high and low growing temperatures can affect plant immunity, and there is evidence that temperature can also influence *P. brassicae* infection, with no infection in canola when temperatures are below 17°C or higher than 26°C (Gossen et al., 2012). Sharma et al. (2011) indicated that low temperature could slow clubroot development in *B. rapa*, therefore temperature should not be a neglected factor when investigating CR mechanisms.

12 | CONCLUDING REMARKS

The clubroot pathogen *P. brassicae* has been in the spotlight for the last decade. Fortunately, this does not seem likely to change soon thanks to the different funding opportunities dedicated to clubroot in European countries, Canada, and China. Based on this extensive

literature review and the work in progress in our laboratories, we predict that four main areas are going to be investigated in the future on the shoulders of omics technologies: (i) identification and functional characterization of clubroot resistance genes that could lead to the generation of broad-spectrum resistance through new technologies like genome editing, (ii) functional characterization of clubroot pathogen effectors and avirulence genes, (iii) identification and characterization of new clubroot biocontrol agents through the investigation of the soil microbiome, and (iv) deepening our comprehension of the host response to the clubroot pathogen during primary and secondary infection, expanding the knowledge previously generated by other studies that have been tackling these areas (Chen et al., 2018; Zhao, Bi, et al., 2017; Zhao, Gao, et al., 2017). Altogether, the goal of the clubroot scientific community is to keep understanding this mysterious pathogen, stop the propagation of the disease, and achieve stable and efficient resistance to clubroot disease. We are confident that new technological advances will help us to understand further the biology of the infection and contribute to the control of this destructive pathogen.

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AUTHOR CONTRIBUTIONS

E.P.L. conceived and designed the work. M.A.J., M.G.G., R.S., N.Z.N., and E.P.L. designed and constructed the figures and graphics. All the co-authors contributed to writing different sections of the manuscript.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

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

ORCID

Edel Pérez-López https://orcid.org/0000-0002-3708-8558

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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