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Genomic approaches for improving resistance to Phytophthora crown rot caused by *P. cactorum* in strawberry (*Fragaria* × *ananassa*)

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Phytophthora crown rot (PhCR) caused by *Phytophthora cactorum* is one of the most damaging soilborne diseases of strawberry in the United States and worldwide. Limitations on fumigants such as methyl bromide have led to deterioration in the control of *P. cactorum* in recent years. The development of resistant varieties is a critical component of the strategy for combatting this soilborne disease. Here, we review the biology of the pathogen, molecular mechanisms of infection, and management of PhCR in strawberry. Recent genomics advances in octoploid strawberry breeding have been able to gain a deeper insight into the genetic architecture of resistance to PhCR and identified the genetic resistance sources for the improvement of strawberry varieties against the pathogen. Quantitative trait loci (QTL) associated with PhCR resistance have been identified and introgressed for breeding of PhCR resistance in cultivated octoploid strawberry (*F.* × *ananassa*). Further characterizing candidate genes and mechanisms of resistance could facilitate incorporating the resistance genes into commercial varieties through genomics-assisted breeding, respectively. In this review, we address important recent advances and progress for genomics-assisted breeding for the resistance to PhCR and the potential use of CRISPR gene editing in cultivated strawberry.

KEYWORDS

DNA marker, marker-assisted selection (MAS), genome assembly, disease resistance (R) genes, CRISPR genome editing, octoploid reference genome

Introduction

Strawberry is one of the important soft fruit crops popular for its color and taste. Besides its attractive color, strawberry has various health benefits such as high in vitamins, minerals, antioxidants, flavonoids, and phenolic acids (Giampieri et al., 2015). The cultivated strawberry (*Fragaria* × *ananassa*) is an allo-octoploid (2n = 8x = 56) and interspecific hybrid originated from a cross between wild octoploid species *F. chiloensis* and *F. virginiana* (Darrow, 1966; Edger et al., 2019). In 2017, the United States produced 1.6 billion pounds (726 million kg) of strawberries, with a total value of approximately $3.5 billion (NASS, 2017). The yield and quality of strawberry fruit are affected by various environmental and biological factors. The common strawberry diseases in Florida include charcoal rot (*Macrophomina phaseolina*), Colletotrichum crown rot (*Colletotrichum gloeosporioides*), Botrytis fruit rot (*Botrytis cinerea*), Phytophthora crown rot (*Phytophthora cactorum*), anthracnose fruit rot (*Colletotrichum acutatum*), powdery mildew (*Podosphaera apahanis*), leather rot (*P. cactorum* and *P. nicotianae*), Phomopsis leaf blight and soft rot (*Phomopsis obscurans*), stem end rot and leaf blotch (*Gnomonia comari*), and angular leaf spot (*Xanthomonas fragariae*) (Oliveira and Peres, 2020).

Among various diseases, economic losses due to root and crown rots are estimated about $150 million per year in the United States (Samtani et al., 2019). In the Florida industry itself, it is estimated that yield loss due to soilborne diseases costs $15 million in revenue each year, despite annual pre-plant soil fumigation (personal communication with Florida Strawberry Growers Association). *Phytophthora* crown rot (PhCR) disease caused by *P. cactorum* is one of the most important soilborne diseases of strawberry in the United States and worldwide, causing up to 40% production losses (Stensvand et al., 1999). This pathogen was first reported in Germany in 1952 (Deutschmann, 1954) and has been problematic in strawberry production worldwide (Schafleitner et al., 2013).

Limitations on fumigants have imposed challenges for the control of *P. cactorum* in recent years. Commercial cultivars vary widely in their genetic resistance to this pathogen, with the most resistant varieties providing high levels of disease control (Mangandi et al., 2017). Thus, the development of resistant cultivars will be a critical component of the strategy for managing this disease in an era of limited fumigants and increases in demand for organic strawberries. Also, soil fumigation before planting is often ineffective in controlling this disease in fruit production fields because the main inoculum source is infected nursery stock (Browne and Bhat, 2011; Baggio et al., 2021). Although various practices together with chemical pesticides are used to manage PhCR, understanding the mechanism of disease resistance and developing disease-resistant cultivars is the best strategy to protect against the pathogen.

Several breeding efforts have been made in the past to identify the genetic resource of resistance for breeding in both diploid and octoploid strawberries (Eikemo et al., 2003; Shaw et al., 2006; Shaw et al., 2008; Eikemo and Stensvand, 2015). The resistance locus Resistance to *Phytophthora cactorum* 1 (*RPc-1*) has been identified in *F. vesca* (Davik et al., 2015). In the octoploid cultivated strawberry, a major resistance locus, *FaRPc2*, located in a linkage group 7D confers resistance against *P. cactorum* (Mangandi et al., 2017). Other quantitative trait loci (QTL), *FaRPc6C*, *FaRPc6D*, and *FaRPc7D*, were also identified in a biparental mapping population for *P. cactorum* resistance in cultivated strawberry (Nellist et al., 2019). *FaRPc2* and *FaRPc7D* are located in the same genomic region, indicating the same resistance QTL. Pathogen populations can be constitutively diversified in response to resistance genes in plants and high levels of diversity and population structure are observed in the potato late blight pathogen, *P. infestans* (Wang et al., 2017). It would be important to understand the population genetic structure of pathogens for crafting durable resistance and addressing the potential breakdown of resistance genes in field conditions. Currently, there is no information available about the pathogenic diversity of *P. cactorum* in U.S. strawberry fruiting fields and plant nurseries. It is not uncommon to observe pathotypes of other *Phytophthora* species in soybean and potato, as these pathogens have rapidly adapted to overcome resistance genes (Witek et al., 2016; Rojas et al., 2017). In addition, a recent study reported that another *Phytophthora* species, *P. nicotianae*, which is closely related to *P. cactorum*, can cause crown rot of strawberry in the United States (Marin et al., 2018). Unfortunately, little is known about how *P. nicotianae* compares to *P. cactorum* with respect to host resistance, especially for *FaRPc2*, which is the major resistance source for strawberry varieties in the United States. The spatial and temporal deployment of resistance genes of *FaRPc2* combined with knowledge of pathogen populations would be a major factor determining broad-spectrum resistance to PhCR in cultivated strawberry.

This review addresses the current knowledge of *P. cactorum* causing crown rot disease and its management, and recent genomic approaches for the breeding of PhCR resistance in cultivated strawberry.

The genus *Phytophthora* and *P. cactorum* causing Phytophthora crown rot disease in strawberry

The genus *Phytophthora* became widely known among plant pathologists after the late blight disease of potato, caused by *Phytophthora infestans*, in the United States, in 1843, and in Europe, in 1845 (Bourke, 1991; Peterson et al., 1992). The

pathogen destroyed Ireland's potato fields during 1845–1846, leading the country to mass starvation with sociological and economic impacts (Large, 1940). The genus was first described by de Bary, in 1876 (Erwin and Ribeiro, 2005). Within this genus, several species are described, some with a narrow host range while others are pathogenic to more than 900 plant species, such as *P. infestans* and *P. cinnamomi*, respectively (Zentmyer, 1980). Moreover, adaptability, such as hybridization and effective dispersal, have enabled *Phytophthora* species to shift hosts, colonize new niches, and become more aggressive and invasive pathogens (Brasier et al., 1999; Goss et al., 2011; Man in 't Veld et al., 2012; Husson et al., 2015).

Taxonomically, the species *P. cactorum* is within the kingdom Stramenopila, phylum Heterokontaphyta, class Peronosporomycetes, order Peronosporales, and family Peronosporaceae. This hemibiotrophic oomycete pathogen has been reported for the first time from rotting cacti (*Cereus giganteus* and *Melocactus nigrotomentosus*) by Lebert and Cohn (1870) in Czechoslovakia. Several synonyms are listed by Erwin and Ribeiro (2005), but *P. cactorum* is the valid name nowadays. Although it occurs worldwide, it is mostly found in temperate regions causing root and collar rots, fruit rots, cankers, leaf blight, wilts, and seedling blights in various hosts (Erwin and Ribeiro, 2005). This pathogen can cause disease in more than 200 plant species from 150 different genera and 60 families (Tucker, 1933; Nienhaus, 1960). In strawberry, *P. cactorum* was first reported to cause leather rot on fruit (Rose, 1924), then crown rot, in 1952 (Deutschmann, 1954). Since then, crown rot of strawberry caused by this *Phytophthora* species has been detected in most of the United States, Europe, and parts of Asia and Africa.

P. cactorum is a homothallic species producing oospores on several natural media (Gallegly and Hong, 2008). Oogonia features are smooth-walled and hyaline (19–38 µm diameter); antheridia are paragynous and often attached close to the oogonial stalk; oospores could be plerotic or aplerotic within a range of 20–26 µm in diameter. Sporangia are papillate, normally borne terminally, with shape ranging from ellipsoidal to spherical (31.4 ± 4.8 × 26.4 ± 4.0 µm) (Oudemans and Coffey, 1991; Erwin and Ribeiro, 2005). Sporangiophores are often simple or in a close or lax sympodium with clustered sporangia (Erwin and Ribeiro, 2005; Abad et al., 2022). Chlamydospores are not very often produced, but when present, they are terminal or intercalary and globose (17–55 µm in diameter). Colonies have no distinct pattern in V8 agar, potato dextrose agar, and malt extract agar with cardinal temperatures of 4°C for minimum, 24°C for optimum, and 30° C for maximum growth (Abad et al., 2022).

P. cactorum shares the Clade 1a of the *Phytophthora* phylogenetic tree based on the internal transcribed spacer (ITS) region with *P. idaei, P. pseudotsugae*, and *P. hedraiandra* (Abad et al., 2022). Its distinguishable features from the other species are the production of caducous sporangia with short pedicels (less than 40 mm) and paragynous antheridia (Erwin and Ribeiro, 2005). Besides morphology, BLASTs of the ITS and cytochrome c oxidase subunit 1 (COI) regions are often used for *Phytophthora* species identification (Robideau et al., 2011). However, a method for routine and rapid detection of *P. cactorum* was created based on high-resolution melting (HRM) assay, which facilitates correct diagnosis, the study of their host ranges and distributions, and accelerates management decisions (Ratti et al., 2019).

Morphology of sporangia and oogonia can slightly vary among *P. cactorum* isolates from different hosts, but due to subtle variations, these characteristics are not enough for isolate differentiation (Hantula et al., 2000). However, some molecular markers can distinguish among isolates from different plant species (Cooke et al., 1996; Hantula et al., 1997; Lilja et al., 1998). Variations can also be observed among isolates from the same host infecting different tissues, such as isolates infecting strawberry crown and fruit, causing crown rot and leather rot, respectively. For a long time, it has been believed that crown rot could only be caused by strains isolated from strawberry crowns or from soil, whereas isolates from other hosts and strawberry fruit could produce leather rot (Seemüller and Schmidle, 1979; Harris and Stickels, 1981; Hantula et al., 2000). Although crown rot is likely caused by a genetically distinct pathotype of *P. cactorum*, isolates have the ability to cause leather rot on strawberry; however, not all isolates from leather rot have the ability to cause crown rot (Eikemo et al., 2004; Nellist et al., 2021). Additionally, genetic separation among isolates of *P. cactorum* in North America and Europe was not obtained with UPGMA analysis; however, host specificity among the strains was observed. Contrastingly, AFLP markers showed that leather rot isolates were different from crown rot isolates during the analysis of 44 isolates of *P. cactorum* from strawberry and other hosts (Eikemo et al., 2004). Besides *P. cactorum*, other species can also be associated with causing crown-rot-like symptoms. *Phytophthora fragariae* causes red stele (red coloration in the core of the root), which can be significant in some strawberry-growing regions leading to severe stunting and mortality. Other species of *Phytophthora* have also been occasionally reported in strawberry, such as *P. citricola*, *P. citrophthora*, *P. eryptogea*, *P. bishii* (= *bisheria*), *P. megasperma*, *P. fragariaefolia*, and *P. nicotianae* (Jeffers and Scott 1953; Meszka and Michalecka 2016; Marin et al., 2018; Farr and Rossman, 2022).

Biology of *Phytophthora cactorum* on strawberry plants

Disease and symptoms

PhCR of strawberry can be caused by several *Phytophthora* species; however, *P. cactorum* is the most important. Early symptoms are characterized by young leaves turning bluish-

green, which eventually begin to wilt. Due to disease development and crown collapse, the whole plant wilts and dies within days (Peres and Baggio, 2019). When the diseased plant is removed from the ground, the crown likely breaks at the upper end. Dark reddish-brown discoloration of crowns starting at the upper or bottom part and disintegration of the vascular tissue are characteristic symptoms of this disease (Figure 1), which could be confused with symptoms caused by other crown rot pathogens, such as *C. gloeosporioides* and *M. phaseolina*. Depending on the environmental factors, the number of crowns within the plant, and cultivar tolerance, the rotting process of the crown may stop leading to recovery or stunting of the plant (Maas, 1998).

Pathogen biology, epidemiology, and disease cycle

Pathogen infection and disease development require specific factors and, if not fulfilled, infections may remain latent, such as in cold-stored plants. Warm weather and abundant wetness trigger disease occurrence and development (Maas, 1998). In strawberry annual production systems in open fields that adopt overhead irrigation after transplanting to promote plant establishment, these conditions are common, leading to outbreaks in the early season of diseases caused by oomycetes (Marin et al., 2019; Baggio et al., 2021). Strawberries are more susceptible at transplanting, and 45 to 60 days later, which correspond to periods when the plant is metabolically more active due to rapid juvenile growth, root development, and fruit production (Marin et al., 2022a). Constant availability of new root growth is a likely cause of the rapid development of damaging *Phytophthora* populations under favorable conditions. Although cultivar resistance is genetically determined, digging immature plants from the nursery could lead to outbreaks in fruit production fields. Moreover, plants under stress are more prone to disease development, such as frigo plants transplanted during warm weather (Lederer and Seemuller, 1992).

The disease cycle may slightly change depending on the strawberry production system (Figure 2). For the perennial production system and nursery fields, due to the constant presence of strawberry plants, the source of inoculum could be oospores that are produced from sexual reproduction between antheridium and oogonium and survived in the soil or plant debris (Srivastava et al., 2020). This survival structure, under conducive conditions, germinates and produces sporangium that contains zoospores. Either the germinated sporangium or the flagellated zoospores can infect the plants, usually through wounds during transplanting. Zoospores move in the water, discard flagella, and synthesize a cell wall, forming a cyst. The cyst germinates and enters host tissue through natural openings like stomata or penetration of host epidermal cells that occurs with the help of an appressorium-like swollen tube (Hohl and Stössel, 1976). Specifically for nursery production, cold-stored plant material (frigo plants) could also serve as the major source of inoculum (Pettitt and Pegg, 1994). The expression of symptoms is linked to the time of planting, since during winter, the pathogen reduces its activity due to low temperatures, delaying disease progress (Maas, 1998). For annual strawberry production, where plants are usually cultivated during the winter, the pathogen might not survive between production seasons, during summer. In Florida, through nurseries monitoring and studies aiming to access the

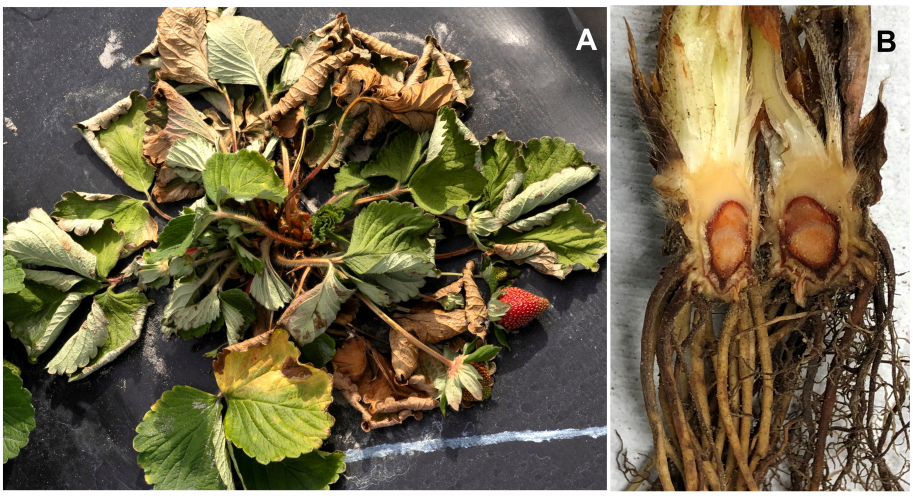

FIGURE 1
Symptoms of Phytophthora crown rot disease in strawberry. **(A)** Strawberry plant wilting, and **(B)** crown rot symptoms caused by *Phytophthora cactorum*.

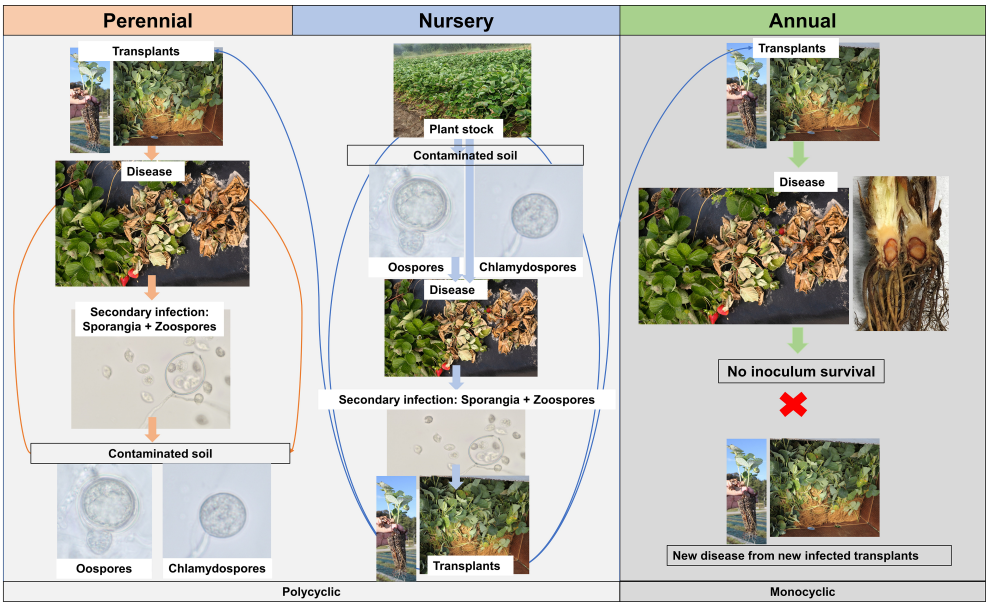

FIGURE 2
Disease cycle of Phytophthora crown rot caused by *Phytophthora cactorum* according to the strawberry production system. For the annual production system, the scheme was drawn based on the findings reported by Baggio et al. (2021).

Phytophthora survival over seasons, the major source of inoculum is likely transplants harboring quiescent infection from nurseries (Baggio et al., 2021); however, this scenario could be different for strawberry growing regions lacking such information. The strawberry propagation system in nursery fields varies, but it is common that at least the third and fourth generations of transplants are multiplied in open fields, being subjected to pathogen infection (Rahman et al., 2015). A similar situation could happen at the nursery level with the exchange of plant stock materials within and among nurseries. After introduction of the pathogen in a field, under wet and favorable conditions, zoospores are produced by infected plants and can disseminate at short distances through free water present in poorly drained soil and penetrate strawberry plants. However, these secondary infections are more likely to occur at the nursery level or in perennial production systems, where the density of plants is higher and plants are maintained in the fields for longer periods, resulting in multiple disease cycles within one crop cycle, characterizing a polycyclic disease. Conversely, in annual production systems, where secondary infections are rare during the same crop cycle, the disease has a monocyclic pattern of development.

Disease management—cultural control

PhCR is usually managed with a combination of chemical and non-chemical methods. Cultural control methods can vary depending on the strawberry region and production system adopted. Studies carried out in Florida, where the annual production system is adopted, show that the pathogen inoculum likely does not survive in the soil and asymptomatic nursery transplants harboring latent infections are considered the major source of inoculum (Baggio et al., 2021). However, the inoculum survival could greatly vary according to weather and soil type among different strawberry growing regions; therefore, more studies aiming to monitor the inoculum survival over time in other areas are needed, such as the one carried out by Baggio et al. (2021). Foremost, the acquisition of free and clean plant stock is detrimental to avoid the introduction of the pathogen and the start of an epidemic. In perennial production systems, where the pathogen can survive in the soil and plant debris, practices that aim to eliminate or reduce the inoculum present in the soil are usually adopted. Among these methods, soil fumigation and soil solarization have been reported to be effective in controlling *Phytophthora* species. Solarization is carried out after the beds are formed and can be effective if weather conditions are ideal (30–45 days of hot weather that promotes soil temperatures of at least 50°C). Also, steam disinfestation could be used as an alternative to chemical fumigation, since raising the soil temperature to 70°C for 30 min to a depth of 0 to 25 cm effectively reduced soilborne diseases (Fennimore and Goodhue, 2016).

Regardless of the production system, PhCR incidence is usually lower in sites with appropriate soil drainage. Improved soil drainage is usually achieved with properly prepared and

tilled soil, preventing the formation of soil layers that are impervious to water. The pathogen could also be introduced in a disease-free area from infested areas through contaminated irrigation water. Therefore, the adoption of appropriate irrigation practices, such as reducing overhead irrigation to avoid free water availability and monitoring water from reservoirs for pathogen occurrence, could avoid the introduction and minimize the dissemination of the pathogen in a field. The adoption of plastic-mulched, raised beds also contributes to disease management. Besides permitting the use of drip irrigation systems, in which low amounts of water are provided to the plants, it improves soil drainage and allows the application of soil fumigants, which can help with inoculum reduction in soils infested with *Phytophthora* species.

To manage diseases in the plant stock materials at the nursery level, thermotherapy of asymptomatic nursery transplants infected with *P. cactorum* could be a good alternative to manage pathogen populations in plant stock. Studies have shown that exposing transplants to aerated steam in a closed chamber at 37°C for 1 h, followed by 44°C for 4 h, was effective in reducing *P. cactorum*, including mefenoxam-resistant populations and disease incidence in production fields (Baggio et al., 2021). The other chemical alternative would be the use of biological products; however, their efficacy may vary across different seasons if environmental conditions are not always conducive to the biological agent (Hautsalo et al., 2016). Some products containing *Streptomyces* spp., *Bacillus subtilis* strain AFS032321, and *Pseudomonas chlororaphis* strain AFS009 were tested at field conditions in Florida and had a positive effect on reducing PhCR (Mertely et al., 2020; Baggio et al., 2021). However, more studies are needed to really access their efficacy across multiple seasons in Florida and different strawberry growing regions.

The adoption of tolerant or less-susceptible cultivars is a desirable strategy and a sustainable method in managing PhCR. Among some of the FL and CA cultivars, 'Sweet Charlie', "Florida Radiance', 'Florida 127' Sensation™, and 'Florida Brilliance' are susceptible, whereas 'Strawberry Festival', 'Florida Elyana', 'Florida Beauty', 'Fronteras', 'Merced', 'Albion', 'San Andreas', and 'Portola' are considered tolerant (Seijo et al., 2020; Marin et al., 2022a). Unfortunately, most cultivars are bred and selected based on fruit yield and quality and not on tolerance to PhCR. Therefore, it falls on breeding programs for the development and deployment of new cultivars with pathogen resistance, fruit quality, and competitive yield.

Disease management—chemical control

In areas where the pathogen may survive in the soil, soil fumigation can provide adequate control of the disease. Nursery and fruit production growers have always relied on soil fumigation using methyl bromide due to its high effectiveness in controlling soilborne pathogens (Santos et al., 2006). However, its phase out in 2013 in the United States and being banned in the EU from 2010 led producers to transition to alternative broad-spectrum fumigants, such as chloropicrin, 1,3-dichloropropene, metam sodium, dazomet, allyl-isothiocyanates, methyl iodide, dimethyl disulfide, and ethanedinitrile, which are usually not as effective (Baggio et al., 2020; Holmes et al., 2020). Limitation of the product movement throughout the soil profile due to its chemical features may jeopardize the reduction of pathogen population in the soil. Regardless of the production system, fungicide applications during transplanting and fruit production are still the predominant control method and, basically, only metalaxyl or mefenoxam and potassium phosphite products are available and registered for strawberry production in the United States (Peres and Baggio, 2019). However, due to the emergence of mefenoxam-resistant isolates and the failure of phosphite products in controlling *P. nicotianae*, management of PhCR has become even more challenging (Marin and Peres, 2021; Marin et al., 2021). Moreover, even resistance to quinone outside inhibitor fungicides has been reported in *P. cactorum* and *P. nicotianae* in Florida (Marin et al., 2022b). Thus, there is a demand for further research to find more effective alternative broad-spectrum fumigants and/or more efficient application methods, fungicides with new modes of action, and alternative biorational products. To improve the management options, alternative oomyticides were investigated and potential products were found; however, they are still not registered for strawberry (Marin and Peres, 2021). Moreover, disease management must be based on integrating the adoption of tolerant cultivars, cultural practices, and chemical applications to minimize the limitations of each control method.

Molecular mechanisms for the pathogen infection in strawberry

Various fungal pathogens use plant cell-wall degrading enzymes like CAZymes and mechanical force to break those barriers to infect and extract nutrients from the host cell. A total of 696 transcripts encoding CAZymes targeting cellulose, hemicellulose, and pectin were identified in the genome of *P. cactorum* 10300 (Armitage et al., 2018). Plant cell wall degrading enzymes, protease inhibitors, and phytotoxins of the PcF Toxin family are the effectors deployed by *Phytophthora* spp. during the infection of the host (Blackman et al., 2014). The delivery of various effector proteins (apoplastic or cytoplasmic) into the host tissue by a virulent pathogen targets plant immune system-related proteins (Dou and Zhou, 2012) and helps them to defeat plant immunity and initiate infection (Dangl et al., 2013). These effectors are secreted into the apoplast or host cells with the help of haustoria (Petre and Kamoun, 2014). In most of the

Phytophthora species, a conserved RXLR motif at the N terminus is contained in one of the important classes of cytoplasmic effectors (Jiang et al., 2008). Various avirulence proteins (RXLR cytoplasmic effectors) like *PiAvr3a, PiAvr2, PiAvr4*, and *PiAvrB1b1* has been localized to haustoria showing it as a site of secretion and delivery into host cells (Birch et al., 2006; van Poppel, 2009; Gilroy et al., 2011). The presence of infection-induced haustorium membrane protein 1 (PiHmp1) is important for infection in potato by *P. infestans* (Avrova et al., 2008). The importance of this protein for infection is highlighted with transient silencing of PiHmp1 that prevented infection to the host tissue.

Transcriptomic profiling of three different life cycle stages (mycelia, zoospores, and germinating cysts with germ tubes) of *P. cactorum* revealed the induction of numerous RXLR and Nep1-like proteins (NLPs) and the downregulation of the majority of Crinklers (CRNs) genes in germinating cysts with germ tube stage (Chen et al., 2018). Since cyst with the germ tube stage is an important pre-infection stage, the upregulated genes play a major role during the infection to host tissue. Moreover, gene expression patterns before and after the infection by *P. cactorum* identified various RXLR effector genes (Chen et al., 2011). Transcriptomic analysis of *P. cactorum* revealed a gene encoding the small cysteine-rich secretory protein SCR96 to be a virulence factor and important for oxidative stress tolerance (Chen et al., 2014). The comparison of transcriptomes of two *P. cactorum* isolates Pc407 (high virulence) and Pc440 (low virulence) obtained from *Fragaria vesca* revealed the high expression of a gene encoding a GAF sensor protein in the high virulence isolate Pc407 that is potentially involved in membrane trafficking processes (Taljoma et al., 2019). Lipid catabolism and elicitins were more highly expressed in Pc407 than in Pc440 at 48 h post-inoculation. The members of the glycosyl hydrolase family 1 involved in the metabolism of glycosylated secondary metabolites were expressed more in Pc407 than in Pc440. In addition, the more virulent isolate Pc407 depends on a smaller set of key RXLR effectors while infecting the host tissue whereas the less virulent isolate Pc440 relies on more RXLR effectors. We propose that *P. cactorum* infection in strawberry would involve a cascade of enzymes and effectors to infect plants similar to the infection mechanism used by other *Phytophthora* species.

Genome sequencing of *P. cactorum* and its application to control Phytophthora crown rot

The exploitation of DNA sequencing helped in learning more about the genetics of *Phytophthora*, species differentiation, phylogeny construction, and grouping into clades (Armitage et al., 2021). The availability of isolate sequences would help to identify similarities and differences in addition to showing if any gene or DNA region is identical (or nearly identical), in which case the isolates belong to the same species. *P. cactorum* infects a wide range of plants including ornamentals, trees, and fruit crops. The whole genome sequencing of *P. cactorum* from *Panax* species revealed its genome size to be 121.5 Mb using third-generation single-molecule real-time (SMRT) sequencing technology (Yang et al., 2018). The genome is highly heterozygous and is the second largest genome within the *Phytophthora* genus. On comparing the genome of *P. cactorum* with other *Phytophthora* spp., it was revealed that *P. parasitica, P. infestans*, and *P. capsici* had the closest relationships (highly syntenic). The extensive expansion of genes encoding detoxification enzymes and carbohydrate-active enzymes (CAZymes) showed comparative analyses of *Phytophthora* genomes. Also, due to the wide host range, *P. cactorum* had higher utilization or detoxification ability against ginsenosides (a group of defense compounds synthesized by *Panax* species) compared to *P. sojae*. A total of 901 and 282 genes were predicted to putatively encode CAZY enzymes in *P. cactorum* from *Panax* and strawberry, respectively (Armitage et al., 2018). Also, tolerance to fungicides in *P. cactorum* involved many proteins in the detoxification metabolic pathway, revealed by proteomic analysis (Mei et al., 2014). The identification of peroxidase (POD), glutathione S-transferases (GST), cytochrome P450 (CYPs), methyltransferase (MTR), dehydrogenase, ATP-binding cassette (ABC) transporter families, and major facilitator superfamily (MFS) in *P. cactorum* genome supports the ability of the pathogen to detoxify secreted ginsenosides (Yang et al., 2018).

Utilizing *de novo* prediction and homology-based comparison, the genome of *P. cactorum* was found to have 56.7 Mb repetitive elements, which constitutes 46.7% of the assembled genome (Yang et al., 2018). Of the 46.7% repetitive elements in the genome, 45.3% include transposable elements (TEs), 20.3% of which include long terminal repeats (LTR). The majority of unique genes in *P. cactorum* were related to defense response, the cell cycle, interaction between organisms, regulation of the cell cycle, TOP signaling pathway, and peptidyl-amino acid modification. The genome sequence of *P. cactorum* isolate 10300 isolated from infected strawberry tissue generated a 59.3-Mb genome assembly with a *de novo* assembly using ABySS (Armitage et al., 2018). The genome size of *P. cactorum* from strawberry is smaller compared to the genome of *P. cactorum* from *Panax* species (121.5 Mb). Also, the percentage of repetitive elements is 18% in the *P. cactorum* genome from strawberry versus 46.7% in the genome from *Panax*. A total of 23,884 genes encoding 24,189 proteins were predicted from its genome, and the number of genes that encoded putatively secreted proteins was 2,234. Although the numbers of genes predicted might be variable within *Phytophthora* species, the greatest number of complete single copy core eukaryotic genes

(CEGs) was obtained in *P. cactorum* gene models, indicating a good representation of gene space within gene models (Armitage et al., 2018).

The predicted genes revealed a plethora of genes encoding putative secreted effectors containing about 200 RxLR domain-containing effectors, 77 CRNs, and apoplastic effectors such as phytotoxins (PcF proteins) and necrosis inducing proteins that play a significant role in infecting the host tissue. Genome sequencing and comparative analysis of 18 isolates of *P. cactorum* revealed evidence for host specialization (Armitage et al., 2021). Isolates causing PhCR in strawberry are globally similar whereas greater diversity was observed for apple-infecting isolates. Discrete lineages and non-recombining clades were observed within *P. cactorum* for specialized strawberry PhCR and apple-specific isolates. Additionally, gain and loss of effector complements were observed in *P. cactorum* phylogeny presenting determinants of host boundaries. Although the genome sequencing and comparative analysis from different research highlight the role of RxLR effectors, CRNs, and apoplastic effectors, not much has been done to characterize their individual role in infecting strawberry. Additionally, understanding the host specificity of these effectors would allow us to target them for resistance breeding in strawberry to PhCR.

In the future, further genome sequencing of *P. cactorum* isolates and *Phytophthora* spp. would allow for a better understanding of the molecular mechanism of infection *via* comparative genomic analysis. It is crucial to identify and understand the effectors involved during infection to understand the molecular disease mechanism. Also, the durability and functionality of the disease resistance depend on the effector protein secreted by plant pathogens, and these isolate-specific effectors could be targeted for resistance breeding in strawberry. Furthermore, the availability of genome sequences for different isolates of *P. cactorum* would help in early detection and surveillance for a new isolate if they evolve in the future and to keep track of different isolates infecting strawberry. The comparative analysis of all the isolates of *P. cactorum* infecting strawberry would additionally provide information about conserved regions across all the isolates. This conserved regions across isolates might serve as the target region in the pathogen genome for resistance breeding against *P. cactorum*.

QTL discovery for the resistance to Phytophthora crown rot in strawberry

The majority of cultivars grown worldwide are susceptible to *P. cactorum*, although high level of resistance was observed in a few cultivated strawberries and numerous diploid strawberry accessions (Parikka, 2003; Eikemo et al., 2010). The diploid wild strawberry *F. vesca* 'Hawaill 4' genotype showed resistance against *P. cactorum*. A major locus, *RPc-1* (Resistance to *P. cactorum* 1), identified from *F. vesca* explained 74.4% of the phenotypic variation for resistance to PhCR (Davik et al., 2015; Toljamo et al., 2016). In addition to the diploid strawberry, different sources of resistance against *P. cactorum* are available in the octoploid strawberry (Eikemo et al., 2003; Shaw et al., 2006; Shaw et al., 2008; Mangandi et al., 2017; Nellist et al., 2019). The testing of 26 cultivars and five selections for resistance to *P. cactorum* crown rot revealed that the genetic background greatly affects the degree of resistance and susceptibility to *P. cactorum* (Eikemo et al., 2003). In another study, 186 genotypes from the University of California strawberry breeding program were evaluated for the resistance to *P. cactorum* and identified that the phenotypic variation of 60.6% for the PhCR resistance was due to the different genetic backgrounds among the genotypes (Shaw et al., 2006). The maximum resistance was observed for 'Albion', 'Aromas', and 'Camino Real', moderate resistance for 'Camarosa', and minimum resistance for 'Diamante' and 'Ventana' (Shaw et al., 2006). Conventional and biotechnological approaches could be used to transfer the source of resistance to susceptible cultivars.

In the strawberry breeding program of the University of Florida, a major locus conferring resistance to PhCR referring to *FaRPc2* was identified using a pedigree-based analysis in complex, multiparental population sets (Mangandi et al., 2017). The resistance locus *FaRPc2* was mapped to linkage group 7D and accounts for most of the genetic variation existing in the breeding population tested for the PhCR resistance. Within the *FaRPc2* locus, it was found that two major haplotypes H2 (*FaRPc2-H2*) and H3 (*FaRPc2-H3*) are highly associated with resistance against *P. cactorum*. The two resistant haplotypes may be originated from different resistance sources, and it is possible that two distinct functional resistance alleles are present at *FaRPc2*. The *FaRPc2-H3* genotype is more prevalent in commercial strawberry varieties in the United States, while *FaRPc2-H2* seems to be more frequently present in European accessions (Noh et al., 2018). However, very little is known about molecular mechanisms of resistance and candidate genes in the *FaRPc2* region. To achieve the long-term goal of achieving durable resistance against *P. cactorum*, it would be important to combine multiple resistance genes and/or alleles. Additionally, three QTLs (*FaRPc6C*, *FaRPc6D*, and *FaRPc7D*) were reported in European germplasm on chromosomes 6-2, 6-4, and 7-3 using biparental population ('Emily' × 'Fenella') inoculated in a greenhouse (Nellist et al., 2019). Three QTLs explained about 37% of the phenotypic variation (Nellist et al., 2019). The significant SNP markers for *FaRPc7D* were located within the same QTL region of *FaRPc2*. Biparental mapping population could detect locus-determining small genetic variability because it includes less genetic and allelic diversity from fewer parents. However, the use of multi-parental populations enabled the exploration of allelic diversity and

increased the accuracy of QTL detection (Mangandi et al., 2017). Thus, QTLs identified using a multifamily population would be effective in diverse genetic backgrounds, whereas QTLs identified using biparental population might be less effective for wide genetic backgrounds.

Genomics-assisted breeding approaches for the resistance against *P. cactorum*

The application of genomics in strawberry breeding has lagged compared to other major crops because of its highly heterozygous allo-octoploid genome, and limited genomic resources in the past. However, the recent availability of reference genome of octoploid strawberry from 'Camarosa', SNP genotyping array (FanaSNP), and high-throughput DNA tests enabled the use of genomic approaches in strawberry breeding (Bassil et al., 2015; Edger et al., 2019; Whitaker et al., 2020; Oh et al., 2020; Liu et al., 2021). In addition, the recent haplotype-phased genomes from University of California variety 'Royal Royce' and University of Florida elite breeding line 'FL15.89-25' shed light on advanced genomics research in strawberry (Hardigan et al., 2021; Fan et al., 2022). Several disease resistance traits are controlled by major loci such as *FaRPc2*, *FaRCa1*, and *FaRCg1* (Mangandi et al., 2017; Salinas et al., 2019; Chandra et al., 2021). In some cases, complex traits may not be controlled by a few loci, and the application of genome-wide prediction would be an alternative strategy for improving traits of interest (Whitaker et al., 2020; Tapia et al., 2022). Genome-wide prediction in strawberry had been used in the past for parent selection of positive yield and quality traits (Petrasch et al., 2022). This approach could be useful for predicting breeding values without phenotypic information (Whitaker et al., 2020).

The development of genetically resistant varieties against *P. cactorum* is the most reliable and long-lasting method to protect strawberry plants from the pathogen. The screening and identification of a durable source of resistance are essential to transfer the source into a susceptible variety. The classical breeding for resistance involved the transfer of *R* genes from one germplasm to another. Breeding efforts for *P. cactorum* resistance started in 1992, and mostly focused on testing methods for susceptibility in mature plants (Simpson et al., 1994). Genomic tools have been used to develop molecular markers tightly associated with candidate *R* genes in the selection of parents and predicting inheritance pattern in progenies for early selection, thus reducing the costs associated with field trials (Akino et al., 2014; Peace, 2017). The development of the first 90K Axiom single nucleotide polymorphism (SNP) array enabled the identification of QTLs associated with the trait of interest to develop molecular markers (Bassil et al., 2015). A compendium of strawberry DNA tests was created and subsequently available to assist the breeders with the molecular markers available for various traits in strawberry (Oh et al., 2020). These DNA tests would help breeders to exploit the genetic resources available in their breeding program to breed better cultivars.

Rapid DNA extraction and high-resolution melting analysis were used for selection at the *FaRPc2* loci conferring *Phytophthora* crown and root rot resistance in the strawberry breeding program at the University of Florida in 2015 (Lee et al., 2016). In addition, the development of high-throughput molecular markers and fine-mapping of *FaRPc2* has been described in the octoploid strawberry (Noh et al., 2018). The high-resolution SNP molecular markers were developed for H2 and H3 haplotypes responsible for resistance to *P. cactorum* on strawberry. A total of seven markers for H2 (*FaRPc2-H2*) and four for H3 (*FaRPc2-H3*) resistance haplotype were tested for the University of Florida strawberry breeding population and other varieties from different breeding programs, respectively (Noh et al., 2018). All the markers were successfully utilized to breed new resistant varieties *via* marker-assisted selection.

Recently, the availability of reference genome and sequencing technologies helped to identify *R* genes associated with disease resistance in strawberry (Edger et al., 2019; Barbey et al., 2019; Shirasawa et al., 2021). The comparison of *R* genes present in cultivated strawberry with their diploid ancestors helped to understand their presence and retention during the transition from diploid to octoploid, and expression pattern (Barbey et al., 2019). The diploid ancestral relatives of the modern cultivated strawberry could possess genetic resources for disease resistance that could be introgressed to susceptible varieties.

Candidate genes and molecular mechanisms for the resistance against *P. cactorum* in strawberry

The *R* gene-related transcripts including receptor-like kinases, nucleotide-binding site leucine-rich repeat (NBS-LRR), and toll/interleukin-1 receptor domain-containing proteins were highly upregulated in the resistant genotype of *F. vesca* compared to the susceptible genotypes (Gogoi et al., 2019). A PCR-based approach (NBS profiling) identified 23 distinct resistant gene analog fragments of NBS-LRR from a resistant genotype of *F. vesca* (Chen et al., 2016). One of the RGAs, RGA109, was characterized as a putative NBS-LRR R protein. *RPc-1* locus (resistant to *P. cactorum*) included one NB-LRR protein that was upregulated in resistant genotypes after the pathogen infection. Similarly, potential resistant genes were also identified in *RPc-1* locus from root transcriptomic analysis of *F. vesca* (Toljamo et al., 2016).

Transcriptomic analysis of *F. vesca* 'Hawaill 4' roots infected with *P. cactorum* revealed potential candidate genes within the QTL region, *RPc1*, responsible for defense (Davik et al., 2015; Toljamo et al., 2016). They identified 69 defense-related genes within *RPc1*; 7 were significantly downregulated, and 19 were upregulated in response to the *P. cactorum* inoculation. Several genes such as NBS-LRR, L-type-lectin-RKLs, and receptor-like protein (RLP) with a leucine-rich-repeat were highly expressed in inoculated plants. The L-type-lectin-RKLs are involved in *Phytophthora* resistance, while LRR-RLPs are associated with various disease resistance in plants (Bouwmeester et al., 2011; Zipfel, 2014; Wang et al., 2015). A phenylalanine ammonia-lyase (PAL) encoding gene in *RPc1* was upregulated after inoculation of *P. cactorum*. The PAL-dependent phenylpropanoid pathway is responsible for salicylic acid synthesis, which is an important hormone signaling pathway associated with fungal pathogen defense responses (Yuan et al., 2009). In addition, WRKY transcription factors located in *RPc1* were significantly upregulated in response to *P. cactorum* inoculation. The transcriptomic analysis of *F. vesca* roots after inoculation with *P. cactorum* provides the possible molecular mechanisms for resistance associated with the pathogen in strawberry. All these RNA-seq transcriptome studies were carried out in diploid *F. vesca*, but none of the gene expression data in response to *P. cactorum* have been reported in the octoploid strawberry.

A number of candidate genes had been identified in plants for *Phytophthora* resistance; however, only limited work has been achieved in the octoploid cultivated strawberry. Analysis of transcriptome data was conducted for the resistance to *C. gloeosporioides* in octoploid strawberry (Wang et al., 2017; Chandra et al., 2021). Chandra et al. (2021) identified several candidate genes for the resistance to Colletotrichum crown rot and developed functional markers linked to the major locus *FaRCg1* conferring resistance against *C. gloeosporioides*. It is important to identify candidate genes and incorporate them into elite breeding materials for developing new resistant cultivars. Expression QTL (eQTL) analysis could also effectively discover gene associations with target traits of interests in strawberry (Barbey et al., 2019). This genomics approach successfully characterized important genes associated with fruit flavors and pathways.

Molecular mechanisms of defense against *P. cactorum* have not been understood in strawberry, although the defense mechanism of various horticulturally important plants against *Phytophthora* spp. is available. The defense system can be categorized as preformed and induced depending on how they respond to the attacking pathogen (Jones and Dangl, 2006). The preformed defense system responds to a different class of pathogens in a similar way. It includes cell wall, waxy epidermal layer, bark, and cuticle that act as the first line of defense used by plants against the pathogens. The induced defense system responds to the virulence factors or elicitors of the pathogen directly or effecting host targets to defend (Van Loon et al., 2006). The first line of defense in strawberry could include cuticle, cell wall, trichomes, and leaf veins against various pathogens such as *B. cinerea, C. acutatum, Tetranychus utricae*, and *Xantomonas fragariae*, respectively (Gooding, 1976; Barritt, 1980; Guidarelli et al., 2011; Steinite and Levinsh, 2003; Kennedy and King, 1962). Biochemical barriers present in various parts of strawberries acts as a defense against various pathogens. The secondary metabolites including glucosinolates, sulfur compounds, phenolics, and saponins form a biological barrier acting at early stage of infection (Kliebenstein, 2004). Salicyclic acid, lignin, and chlorogenic acid are products of the phenylpropanoid pathway and serve as barriers for pathogens in strawberry. A biochemical compound similar to phytoalexin acts to make strawberries resistant to *P. fragariae* (Mussell and Staples, 1971), whereas the presence of antifungal compounds in the strawberry leaves makes it resistant to *Colletotrichum fragariae*. The preformed compound had antifungal activity 15 times more in the moderately resistant cultivar 'Sweet Charlie' than in the susceptible cultivar 'Chandler' (Terry, 2002).

Pattern recognition receptors (PRRs) in the plant cell membrane expose their pathogen/microbe/damage-associated molecular pattern (PAMP/MAMP/DAMP) recognition domains into the apoplast that recognizes conserved oomycete PAMPs and trigger PAMP-triggered immunity (PTI) (Fawke et al., 2015). The recognition of pathogen's effectors entering the host cells mediated by intercellular disease resistance proteins (*R* genes) led to effector-triggered immunity (ETI). PTI is generally measured or marked based on ion fluxes, mitogen-activated protein kinase (MAPK) cascades, production of reactive oxygen species (ROS), cell wall reinforcement, pH alkalinization, callose deposition, and defense gene activation mostly regulated by WRKY transcription factors whereas ETI causes visible controlled cell death known as hypersensitive response (HR) (Ingle et al., 2006). Figure 3 illustrates the potential role of the PAMP- and DAMP-mediated defense pathway against *P. cactorum*. Numerous DAMPs had been identified in plants; however, their roles in plant immunity and defense are not fully understood. Plants could monitor the cuticle integrity and cell walls through DAMPs and activate defense responses. For example, Arabidopsis leaves treated with cutinase or pathogen expressing cutinase gene display strong resistance against necrotrophic fungus *Botrytis cinerea* (Chassot et al., 2007). Oligogalacturonic acid responses were enhanced with overexpression of wall associated kinase 1 (WAK1) and increased resistance to *B. cinerea* (Brutus et al., 2010). Similarly, xyloglucan oligosaccharides act as DAMP and induce immune responses like MAPK activation, callose deposition, and immune gene expression in grapevine and *Arabidopsis* for resistance against *B. cinerea* and *Hyaloperonospora arabidopsidis* (Claverie et al., 2018). Additionally, extracellular amino acid histidine acts as DAMP and induces resistance against soilborne bacterial pathogen

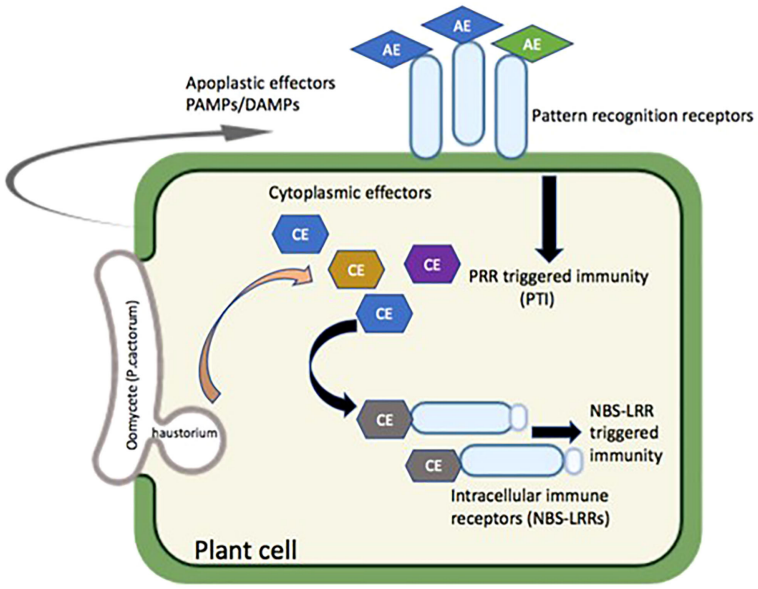

FIGURE 3
Possible mode of action for the resistance against *P. cactorum* in strawberry. PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; AE, apoplast effectors; CE, cytoplasmic effectors; PRR, pattern recognition receptors; NBS-LRRs, nucleotide binding site-leucine rich repeats.

Ralstonia solanacearum and *B. cinerea* in Arabidopsis and tomato, respectively, through activation of the ethylene signaling pathway (Seo et al., 2016). Moreover, the genus *Phytophthora* induces necrosis and immune response in several plant species using cellulose binding elicitor lectin (CBEL). A receptor-like kinase BAK1 (general regulator of basal immunity) is required for CBEL-induced defense responses and respiratory burst oxidase homologue (RBOH)-mediated production of reactive oxygen burst (Larroque et al., 2013). The result showed that a BAK1- and RBOH-dependent CBEL-triggered immunity is important for *Phytophthora* resistance, whereas susceptibility to *Phytophthora* does not correlate with natural quantitative variation of basal immunity triggered by conserved elicitors such as CBEL.

Potential application of CRISPR gene editing for the PhCR resistance in strawberry

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system has been widely applied for targeted gene editing in humans, animals, and plants (Yang et al., 2013; Shalem et al., 2014; Zhang et al., 2014). In plants, genome editing can be used not only for studying gene functions but also in developing new germplasm by inducing mutations or modifying target genes. The method of gene editing has a great potential to enhance the breeding process for improving fruit quality and disease resistance in strawberry (Abdelrahman et al., 2018; Yang et al., 2013; Ito et al., 2015). In traditional breeding, the linkage drag is often challenging for the introgression of new resistance traits from wild varieties into elite cultivars. Because certain resistance genes could be genetically linked to loci associated with agronomically important traits such as yield and plant development (Lewis et al., 2007; Miah et al., 2013; Summers and Brown, 2013), modifying target genes precisely using CRISPR-guided mutation could be the effective way to resolve this issue of linkage drag.

The technique of CRISPR/Cas9 has been applied to improve cultivars for disease resistance against soilborne pathogens in plants (Table 1). Mutations in *Non-expressor of Pathogenesis-Related 3* (*TcNPR3*) gene induced by transient expression of CRISPR/Cas9 lead to enhanced defense response to *P. tropicalis* in *Theobroma cacao* (Fister et al., 2018). *TcNPR3* mutagenesis by CRISPR/Cas9 reduced lesion sizes in CRISPR/Cas9-treated leaf tissues and elevated the expression of pathogenesis-related (PR) genes (Fister et al., 2018). In another study, CRISPR/Cas9-induced mutation in *Downy Mildew Resistance 6* (*DMR6*) enhances defense response to broad-spectrum plant pathogens (*Xanthomonas* spp., *Pseudomonas syringae*, and *P. capsica*) in tomato (de Toledo Thomazella et al., 2016). *SlDMR6-1* mutagenesis by CRISPR/Cas9 delayed disease progression of *P. capsica* and the disease symptom was less severe in *SlDMR6-1* edited plants (de Toledo Thomazella et al., 2016). Furthermore, CRISPR/Cas9-mediated gene editing could precisely

TABLE 1 Applications of CRISPR/Cas9 for improvements of disease resistance to soilborne pathogens.

Plant	Pathogen	Target gene	Reference
Theobroma cacao	*Phytophthora tropicalis*	*TcNPR3*	(Fister et al., 2018)
Solanum lycopersicum	*Xanthomonas* spp., *Pseudomonas syringae*, and *Phytophthora capsica*	*SlDMR6-1*	(de Toledo Thomazella et al., 2016)
Citrullus lanatus	*Fusarium oxysporum*	*Clpsk1*	(Zhang et al., 2020)
Triticum aestivum	*Fusarium graminearum*	*TaNFXL1*	(Brauer et al., 2020)
Gossypium hirsutum	*Verticillium dahliae*	*Gh14-3-3d*	(Zhang et al., 2018)
Brassica napus	*Verticillium longisporum*	*BnCRT1a*	(Pröbsting et al., 2020)

repair mutations on target genes by homologous recombination (HR). Single-nucleotide polymorphisms and small InDel mutations of resistance genes could be potential candidates for CRISPR/Cas9-mediated HR applications. With more knowledge on host–pathogen interactions for PhCR resistance, we would have more feasible options for CRISPR-guided resistance breeding to PhCR in strawberry.

CRISPR/Cas9-mediated target mutagenesis studies have recently been reported in octoploid strawberry (Xing et al., 2018; Martín-Pizarro et al., 2019; Wilson et al., 2019). Transient assay of CRISPR/Cas9 introduced targeted mutations was performed with *FvMYB10* (R2R3 MYB transcription factor 10) and *FvCHS* (chalcone synthase) genes involved in fruit color development. *Agrobacterium*-mediated transformation was utilized for the stable integration of CRISPR/Cas9 construct targeting *PDS* (phytoene desaturase). In addition, the CRISPR/Cas9 gene-editing system was first evaluated for the functional change of gene using *Tomato MADS box gene6* (*TM6*) in the octoploid cultivated strawberry. The *tm6* mutant lines exhibited morphological abnormalities in anthers and pollen grains (Martín-Pizarro et al., 2019).

Because of the high complexity of polyploid genome of strawberry, designing a single guide RNA (sgRNA) specific to a target gene is necessary to reduce any off-target effects. The 5'-end 20 nucleotide sequences in sgRNA determine target specificity and potential off-targets. Particularly, 8–12 nucleotides proximal to PAM (seed sequence) have a crucial role in the target gene recognition and cleavage (Jiang et al., 2013). A number of bioinformatic tools are currently available for designing sgRNA in different plant species (Table 2). CRISPOR, CRISPRdirect, CRISPR-P, CRISPR RGEN Tools, and CHOPCHOP provide a genome-wide survey of specific sgRNAs in diploid (*F. vesca*) and octoploid strawberry (*F. ×ananassa*). Moreover, CRISPR RGEN Tools and PolyOligo provide designing sgRNA on diploid and octoploid strawberry genomes. The recent haplotype phased genome of octoploid strawberry, FaRR1 (cv. Royal Royce), is now available in CHOPCHOP.

Conclusion

The recent genomic advances in both *P. cactorum* and strawberry have been greatly valuable for the resistance breeding of PhCR in octoploid cultivated strawberry. Because of the availability of high-quality chromosome-scale reference genomes of octoploid strawberry, it could be possible to discover more genes and rare alleles associated with the resistance to multiple pathogens and other

TABLE 2 List of sgRNA design programs publicly available.

Program	URL	Strawberry genome
Breaking Cas	http://bioinfogp.cnb.csic.es/tools/breakingcas	N.A.[a]
CCTop	http://crispr.cos.uni-heidelberg.de	N.A.
CGAT	http://cbc.gdcb.iastate.edu/cgat	N.A.
CHOPCHOP	http://chopchop.cbu.uib.no	*Fragaria × ananassa*
CRISPOR	http://crispor.tefor.net	*Fragaria vesca*
CRISPRdirect	http://crispr.dbcls.jp	*Fragaria vesca*
CRISPR-GE[d]	http://skl.scau.edu.cn	N.A.
CRISPR-P	http://cbi.hzau.edu.cn/CRISPR2	*Fragaria vesca*
CRISPR-PLANT	https://www.genome.arizona.edu/crispr	N.A.
CRISPR RGEN Tools	http://www.rgenome.net	*Fragaria vesca* *Fragaria × ananassa*
E-CRISP	http://www.e-crisp.org/E-CRISP	N.A.
GT-Scan	https://gt-scan.csiro.au	N.A.
PolyOligo	http://ec2-52-52-41-39.us-west-1.compute.amazonaws.com/	*Fragaria × ananassa*

[a]Not available.

important breeding characteristics. Furthermore, genomics research for characterizing gene functions will accelerate to develop gene-specific markers and facilitate the effectiveness of marker-assisted selection in development of new disease-resistant varieties. In new approaches of disease resistance breeding, one of the most exciting prospects would be to discover susceptibility (S) genes using an octoploid strawberry pangenome and utilize them with CRISPR/Cas9-guided mutation tools including a DNA-free gene editing platform suitable for generation of breeding accessions and varieties.

Author contributions

SS, MM, NP, and SL developed ideas and prepared the manuscript. SS, MM, ML, and JB wrote the manuscript, and all the remaining authors contributed to the final version of the manuscript.

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Conflict of interest

Author JB completed this manuscript at University of Florida and is now employed by Syngenta Crop Protection, LLC.

The remaining 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.

Publisher's note

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