

MOLECULAR MECHANISMS OF VIRULENCE IN PHYTOPATHOGENIC FUNGI

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ABSTRACT

Fungi are versatile organisms that play diverse ecological roles, acting as decomposers, mutualists, or plant pathogens. This chapter addresses the virulence mechanisms employed by phytopathogenic fungi, which include the adhesion and colonization of plant tissues, invasion by means of hydrolytic enzymes, and the formation of biofilms. Cellular communication via *quorum sensing regulates* processes such as sporulation and morphological transition, while the production of toxins and virulence factors, such as enzymes and secondary metabolites, interferes with the physiological processes of plants, facilitating infection. Fungi also develop sophisticated strategies to evade the plant immune system, such as cell wall modification and secretion of effectors that suppress defense responses. Antifungal resistance and genetic regulation of virulence are discussed, highlighting the importance of integrated control strategies, such as the use of resistant cultivars and biocontrol agents. Advanced diagnostic methods, such as PCR and next-generation sequencing, are presented as essential tools for the early detection and management of fungal diseases.

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1 INTRODUCTION

Fungi are present in practically all environments, acting in the decomposition of organic substrates and playing different ecological roles, such as saprophagous, mutualistic, commensal or pathogenic. As for cell organization, these organisms vary between unicellular forms (such as yeasts) and complex multicellular structures (such as filamentous fungi). Reproductive mechanisms include both asexual multiplication (by budding or fragmentation) and sexual processes, involving hyphal fusion and genetic recombination (Oliveira; Starck; Oliveira, 2021).

Fungi adopt several strategies to colonize plants, resulting in interactions that can be beneficial or lethal to the host. Fungal pathogens are generally classified as biotrophs, which live in plant tissues without killing them, or necrotrophs, which destroy cells to obtain nutrients. There are also hemibiotrophs, which start out as biotrophs and then become necrotrophs. While necrotrophs cause necrosis and can lead to plant death, biotrophs, despite their more subtle symptoms, include some of the most harmful diseases to agriculture (Doehlemann *et al.*, 2017).

Fungi represent the most numerous group of pathogens that affect plant roots, for example. They are present in several agricultural systems and can cause diseases in crops of great economic and social importance. These root fungal infections generate a wide variety of symptoms and are among the main factors responsible for the reduction in agricultural productivity, especially due to their persistent and destructive impact (Agápto *et al.*, 2021). Once present in cultivation areas, these fungi represent a great challenge for management, as their resistance structures remain viable in the soil for long periods. Among them, the microsclerotia of *Verticillium dahliae* and *Macrophomina phaseolina*, the sclerotia of *Sclerotinia sclerotiorum*, *Sclerotium rolfsii* and *Rhizoctonia* spp. stand out, in addition to the chlamydospores of *Fusarium oxysporum* (Bueno; Ambrose; Souza, 2007).

Fungi are also responsible for the emergence of anthracnose. Tropical fruits are one of several crops vulnerable to infection by species of *Colletotrichum*, which causes the disease. Contamination usually occurs in the field during flowering and fruiting, being influenced by factors such as humidity, temperature, fruit condition and inoculum concentration. In some situations, the entry of the fungus can occur directly through natural wounds or openings (Zakaria, 2021). Another crop affected by anthracnose is soybeans, which can cause significant losses in productivity and crop quality. Its control involves different strategies, including the application of fungicides, the cultivation of resistant varieties, and the implementation of appropriate agricultural practices (Reis; Dick; Lima, 2023)

Large-scale agricultural production relies primarily on the use of chemical fungicides to protect cultivated plants against fungal pathogens by eliminating or inhibiting their cells and spores. However, due to their low cost and ease of application, these products are often used excessively or repetitively. This indiscriminate use poses risks to human and animal health and the environment. In addition, the resistance acquired by some strains of phytopathogenic fungi has made it even more difficult to control these diseases. In view of this scenario, safe, sustainable and ecologically correct alternatives, known as green strategies, have been studied as a viable solution for the management of fungal infections in plants (El-Baky; Amara, 2021).

2 ACCESSION AND COLONIZATION

In order to have the adhesion of fungi to their hosts, the cell wall will have a composition that facilitates this process. The cell wall has high plasticity, giving them strength and protection. Fungal cells are made up of carbohydrates (80 - 90%), proteins (5 - 20%) and lipids (1 - 7%), where carbohydrates will be more present in the form of polysaccharides and glycoconjugates (Teixeira, 2011). Proteins, on the other hand, also present in the cell wall, will allow cells to recognize themselves and form a biofilm, growing as a pseudohyphae, in an invasive way (Klis; Boorsma; De Groot, 2006).

The adhesion of fungi to their hosts consists of carrying out some steps. Adhesion begins at the first barrier of the plants, represented by the cuticle, with the growth of the germ tube on this surface (Rhoden *et al.*, 2019). The ability to adhere to inert materials would be an indication of adherence to living cells (Silva *et al.*, 2021). It does not seem to have a pattern of fungal adhesion to its hosts, this process is commonly performed in hydrophobic regions, or mucosal surfaces (Rhoden *et al.*, 2019).

According to Jesus (2013), biofilm is a set of microorganisms that live in association, they group together to form a multicellular community under tissues. Biofilm is a porous and absorbent structure, composed mainly of water, the microorganisms that form biofilm represent less than 10% of its composition (Langer *et al.*, 2018). The development of biofilm involves five steps: (I) the interaction of cells with microorganisms on the surface; (II) its adhesion to the surface; (III) the formation of microcolonies in the upper layer of the hyphae and the production of extracellular matrix by the cells of the pathogenic fungus; (IV) cell maturation; (V) and the formation of biofilm (Jesus, 2013).

The colonization of fungi occurs when the secretion of hydrolytic enzymes occurs and they end up breaking the cuticle, then leading to the colonization of the host's internal tissues, also leading to the plant's organs, and the death of the host (Rustiguel *et al.*, 2018). This

colonization of fungi is facilitated mainly in seeds, when they have high levels of proteins, carbohydrates and minerals, extending colonization to the early stages of seed development (Parisi *et al.*, 2019).

3 INVASION AND DISSEMINATION

3.1 SIGNAL EXCHANGES AND QUORUM SENSING COMMUNICATION

The phenomenon of *quorum sensing* (QS) is a mechanism of cellular communication that has been widely investigated in bacteria and, more recently, in fungi (Kugler *et al.*, 2000). This system regulates the gene expression of microbial populations in response to cell density, coordinating essential collective behaviors, such as biofilm formation, sporulation, and morphological transition (Fuqua *et al.*, 1994; Bandara *et al.*, 2012).

In the fungal context, QS is mainly associated with the dimorphic transition, a process that determines the virulence of pathogens such as *Candida albicans* and *Paracoccidioides brasiliensis*. Farnesol, a sesquiterpene alcohol, stands out as one of the main molecules involved in this mechanism, playing a regulatory role in fungal morphogenesis and exhibiting natural antifungal properties (Hornby *et al.*, 2001; Ramage *et al.*, 2002).

The modulation of the dimorphic transition by farnesol and its impact on the morphology of pathogenic fungi indicate that QS inhibition may represent an effective strategy to prevent the formation of biofilms (Hornby *et al.*, 2001; Jabra-Rizk *et al.*, 2006; Bandara *et al.*, 2012; Brilhante *et al.*, 2013). Although the exact mechanism of action of farnesol is not yet completely elucidated, evidence suggests that its interaction with the fungal cell membrane compromises the synthesis of ergosterol, affecting the structural integrity of the microorganism (Jabra-Rizk *et al.*, 2006; Derengowski *et al.*, 2009).

In addition to its effect on inhibiting fungal growth, studies conducted by Navarathna *et al.* (2007) indicate that excessive secretion of farnesol during infection may increase the virulence of *Candida albicans*. This phenomenon occurs due to the alteration in the fluidity of the host cell membrane, facilitating tissue invasion and resulting in a significant increase in mortality in murine models.

QS plays a central role in the formation and maturation of biofilms, being mediated by inducing or inhibiting signaling molecules that accumulate in the environment, promoting the structural organization of the biofilm (Wongsuk; Pumeesat; Luplertlop, 2016). In more advanced stages, QS regulates biofilm homeostasis, controlling processes such as extracellular matrix production, cell maturation, and dispersion (Mondal; Majumdar, 2019).

In recent years, QS has been considered a strategic target for antimicrobial therapies, due to its involvement in regulating the expression of virulence factors. Given that the

formation and maturation of biofilms are processes dependent on this system, their modulation, either by inactivation or addition of signaling molecules, can significantly alter the dynamics of these structures, facilitating their destabilization and removal (Brackman; Coenye, 2015; Mehmood *et al.*, 2019; Paluch *et al.*, 2020).

3.2 EVASION OF THE IMMUNE SYSTEM

Phytopathogenic fungi have developed a series of sophisticated molecular mechanisms to evade the immune system of plants, ensuring their survival and colonization of host tissues. The plant immune response is based on the recognition of pathogen-associated molecular patterns (PAMPs), activating defense pathways to contain infection (Blanco; Garcia, 2008). However, many fungi are able to modify their cell wall or secrete effector molecules that suppress these responses, allowing them to spread within the plant (Geoghegan, Steinberg & Gurr, 2017).

One of the main mechanisms used by fungi to evade immune recognition is the modification of the composition of the cell wall. The fungal wall contains polysaccharides such as chitin and β -glucans, which are targets of the plants' immune system. However, some pathogens are able to hide or modify these molecules to avoid detection. According to Geoghegan, Steinberg and Gurr (2017), the deacetylation of chitin to chitosan reduces the activation of pattern recognition receptors (PRRs) in plants, minimizing the initial immune response.

In addition to cell wall modification, phytopathogenic fungi produce effector proteins that directly inhibit plant immune responses. These proteins are secreted in the apoplast or within host cells and have the ability to block signaling pathways involved in plant defense. Ferreira *et al.* (2006) point out that fungi such as *Magnaporthe oryzae* and *Botrytis cinerea* produce effectors that suppress the production of reactive oxygen species (ROS), an essential mechanism to contain infections.

Another very important way to escape the plant's immune system is how fungi can alter plant hormones; this makes the host's defenses weaker. Some fungal enemies release substances that mess with the signals of jasmonic acid and salicylic acid; these are two hormones that matter a lot to initiate immune responses in plants (König *et al.*, 2021). By altering these pathways, fungi decrease the level of defense genes that are being expressed, which helps them take control of plant tissues more easily.

Immune evasion is additionally supported by the development of infection structures. Haustoria are intra-host extensions created for nutrient uptake and direct interaction with the plant cell; Some fungi produce these. In addition to their role in effector transfer to inhibit host

defense responses, these structures also function as a means for fungi to evade detection by the host's immune system (Ferreira *et al.*, 2006).

In addition to the strategies discussed above, there is also immune evasion due to the formation of biofilm and resistance to environmental stresses. As stated by Hernández-Chávez *et al.* (2017), biofilm development provides a protection against antimicrobial compounds produced by plants and penetration of these pesticides from agriculture. This adaptation allows fungi to persist in the environment and reinfect new hosts over time.

Due to the complexity of immune evasion by phytopathogenic fungi, their strategies remain poorly understood and therefore urgently needed for effective control methods against plant diseases. The interactions between fungi and the immune system of plants have led to the creation of resistant cultivars and improvements in fungicides. Future studies may seek agricultural management plans specifically targeting some molecular constituents within pathogens to interfere with the survival tactics of these infectious agents.

4 TOXIN PRODUCTION AND VIRULENCE FACTORS

4.1 TOXINS

The production of toxins by pathogenic fungi represents one of the main molecular mechanisms of virulence employed during plant infection. These toxins are specialized secondary metabolites, capable of directly interfering with the physiological processes of the host, facilitating the colonization and dissemination of the pathogen. According to the classification proposed by Walton (1996), fungal toxins can be divided into two main groups: host-specific toxins (HSTs), which affect only certain plant species, and non-host-specific toxins (non-HSTs), which exert toxic effects on a wide range of hosts (Walton, 1996; Dehghi *et al.*, 2015).

Host-specific toxins are considered determinants of pathogenicity, since their production is directly related to the ability of the fungus to infect specific hosts. A notable example is the T-toxin toxin, produced by *Cochliobolus heterostrophus*, which induces the formation of necrotic lesions in corn carrying the *T-urf gene13*. This highly specific interaction is mediated by receptors present in the plant, which recognize and respond to the toxin, triggering a cascade of events that culminate in cell death. Another example is the HC toxin, produced by *Alternaria alternata*, which affects citrus plants, causing the disease known as brown spot. These toxins are often encoded by genes located in *gene clusters, which are coordinated in response to environmental and host signals* (Baker *et al.*, 2006; Walton, 2006; Van Der Does; Rep, 2007; Wu *et al.*, 2012).

On the other hand, non-host-specific toxins have a broader spectrum of action, affecting a variety of plant species. Fumonisin, produced by *Fusarium verticillioides*, are a classic example of this group. These toxins work by inhibiting the enzyme ceramide synthase, which leads to the accumulation of sphingoidins and the destabilization of the plasma membrane of plant cells. In addition, fumonisins induce the production of reactive oxygen species (ROS), causing oxidative stress and widespread cellular damage. Another example is aflatoxin, produced by *Aspergillus flavus*, which inhibits protein synthesis by binding to ribosomal RNA, compromising cell function and facilitating the invasion of plant tissue (Munkvold; Desjardins, 1997; Kimura *et al.*, 2007).

The mechanisms of action are extremely diverse and include inhibition of essential enzymes, induction of oxidative stress, and interference with plant cell signaling. For example, trichothecenes, such as deoxynivalenol (DON) produced by *Fusarium graminearum*, act as translation inhibitors by binding to the 60S subunit of ribosomes, blocking protein synthesis and inducing cell death. In addition, some toxins, such as cercosporin, produced by *Cercospora* spp., act as photosensitizers, generating free radicals under visible light, which causes irreversible oxidative damage to plant cells (Kimura *et al.*, 2007; Proctor; Hohn; McCormick, 1995; Koh *et al.*, 2023).

The production of toxins is closely linked to genetic and environmental regulation. Genes involved in the biosynthesis of toxins are often organized into gene clusters, which are regulated in a coordinated manner by specific transcriptional factors. For example, the *TRI5* gene, which encodes the enzyme trichodiene synthase, is essential for the production of trichothecenes and is regulated by factors such as *TRI6* and *TRI10*. In addition, environmental factors, such as humidity, temperature, and nutrient availability, significantly influence the expression of these genes. Epigenetic regulation, mediated by histone modifications and DNA methylation, also plays a crucial role in modulating toxin production (Walton, 1996; Woloshuk; Prieto, 1998; Kimura *et al.*, 2007).

The interaction between these substances and the plant's response is a key aspect of pathogenicity. Plants have defense mechanisms that include the recognition of pathogen-associated molecular patterns (PAMPs) and the activation of immune responses. However, many toxins act as effectors that suppress these responses, allowing the fungus to evade detection and colonize plant tissue. For example, the toxin victorin, produced by *Cochliobolus victoriae*, induces programmed cell death in susceptible plants, but also suppresses the production of phytoalexins, which are antimicrobial compounds produced by the plant in response to infection (Turgeon; Lu, 2000; Kessler *et al.*, 2020).

The study of these toxins also has practical applications in the development of disease management strategies. The identification of genes involved in the synthesis of toxins and the understanding of their mechanisms of action can support the creation of resistant plant varieties through genetic improvement or gene editing techniques. In addition, the use of microbial antagonists that inhibit toxin production represents a promising approach for the biological control of fungal pathogens (Bhaat; Vasanthi, 1999).

4.2 VIRULENCE FACTORS

Pathogenic fungi are eukaryotic organisms that have the ability to cause disease in human, animal, and plant hosts. The virulence of these microorganisms is associated with a series of factors that give them the ability to adhere, invade, survive and proliferate in the host's tissues, in addition to evading or modulating the immune system. Among the main virulence factors are thermotolerance, dimorphism, cell wall or capsule composition, and the production of hydrolytic enzymes (Kurokawa, 1998; Reviello, 2024).

Thermotolerance is the ability of some pathogenic fungi to grow at 37°C, the temperature of the human body, which is essential for their survival and proliferation in the host. Fungi such as *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum* and *Paracoccidioides brasiliensis* demonstrate this characteristic, allowing them to cause systemic infections in humans (Kurokawa, 1998; Mattoon *et al.*, 2021).

Dimorphism refers to the ability of certain fungi to switch between two distinct morphological forms: the yeast form and the filamentous (hyphae) form. This transition is often triggered by environmental changes, such as temperature or nutrient availability, and is associated with pathogenicity. For example, *H. capsulatum* and *P. brasiliensis* exhibit dimorphism, allowing them to adapt to different environments in the host and facilitate tissue invasion (Walker, 2017; Chaves, 2021).

The presence of specific components, such as capsular polysaccharides in *C. neoformans*, contributes to phagocytosis resistance and host immune response. In addition, the production of hydrolytic enzymes, such as proteases, phospholipases, and elastases, allows fungi to degrade components of host tissues, facilitating the invasion and spread of infection (Karkowska-Kuleta *et al.*, 2009; Mcquiston; Poeta, 2010).

Biofilms are communities of fungi covered by an array of extracellular substances that can form on biological surfaces, such as catheters or internal organs. They represent a form of resistance to antimicrobial treatments and the host immune response. For example,

Candida albicans is known for its ability to form biofilms, which contributes to its pathogenicity and resistance to available antifungals (Ryder, 2001; Goudarzi *et al.*, 2021).

Some fungi produce mycotoxins that can cause direct damage to host tissues or modulate the immune response. For example, species of the genus *Aspergillus* produce aflatoxins, highly toxic and carcinogenic compounds that can contaminate food and cause serious illness in humans and animals (Awuchi *et al.*, 2021).

Evasion of the immune system is critical for the survival of pathogenic fungi in the host. Mechanisms such as phenotypic variation, which involves changes in the expression of surface antigens, allow fungi to avoid recognition by the immune system. In addition, the production of enzymes that degrade effector molecules of the immune system or the modulation of the inflammatory response contribute to the persistence of infection (Dühring *et al.*, 2015; Brown, 2023).

Understanding these virulence factors is essential for developing effective strategies for diagnosing, preventing, and treating fungal infections. Advanced diagnostic methods, such as molecular Polymerase Chain Reaction (PCR) techniques and genetic sequencing, allow for the rapid and accurate detection of pathogenic fungi, facilitating the implementation of targeted antifungal therapies and reducing the morbidity and mortality associated with these infections (Kidd *et al.*, 2020; Pham *et al.*, 2024).

5 RESISTANCE TO ANTIFUNGALS

Factors associated with fungal resistance include gene mutations, migrations, and recombinations. These factors may contribute to the introduction of resistance genotypes in a fungal population (Brent; Hollomon, 2007). The use of fungicides in agriculture, such as azoles, can affect not only phytopathogenic fungal species, but also susceptible species of saprophytic fungi, many of which play a beneficial role in the regulation of pathogenic species (Liggitt *et al.*, 1997).

Micronutrients and improved soil structure increase the soil's ability to sustain biological activity, including organisms that aid in the suppression of phytopathogens. However, it depends on the type of pathogen and the characteristics of the soil (Bettiol *et al.*, 1991). Phytopathogenic fungi that inhabit the soil have specialized resistance structures as their main form of survival under unfavorable conditions. Teliospores, ascocarps, spores, sclerotia and chlamydospores are examples of these specialized resistance structures (Amorim, 1995).

There are viruses capable of favoring the defense mechanisms of plants against phytopathogenic fungi. In their study, Xu *et al.* (2016) reported that *Bacillus velezensis* can

produce enzymes with antifungal activity. Among them, the most found in the literature are lipopeptides, such as surfactins, iturines, and phengyncins (Yu *et al.*, 2002; Ji *et al.*, 2013; Velândia, 2018). The mycovirus can reduce the production of pigments in the fungus, such as melanin (Nuss, 2005), which confers resistance to the fungus, protecting it from antifungals. Almost all the molecules that make up the cell wall of fungi are important or essential for fungal growth or virulence (Gow; Throbbing; Munro, 2017).

6 GENETIC REGULATION OF VIRULENCE AND CHANGES IN METABOLISM

Although fungi are one of the largest groups of plant parasites, most fungi are not pathogenic, and most plants are resistant to fungal infections. However, some species have been developing the ability to cause infection in certain hosts, demonstrating specificity in the plant-pathogen interaction. Advances in research have elucidated some aspects of virulence caused by fungi, highlighting the role of interactions between effectors produced by the fungus and host receptors, biosynthesis of secondary metabolites, signal transduction/cell metabolism, cell trafficking/secretory system, and the channeling of non-coding RNAs (John *et al.*, 2021). Virulence in fungi is often associated with genes encoding avirulence factors and proteins that act as host-specific toxins. Several genes involved in virulence have been identified in pathogenic fungi. Virulence-related gene sequences often exhibit significant polymorphism, which may be indicative of rapid adaptation to selective pressures from host plants, especially under growing conditions where new resistant varieties are introduced. The role of avirulence factors in determining host specificity suggests complex interactions between pathogen genes and plant resistance genes. Modifications in avirulence genes can lead to new strains of fungi that evade recognition by plants, resulting in a phenomenon called "virulence evolution" (Li; Cornelissen; Rep, 2020). Most of the genes encoding effectors and secondary metabolites responsible for the development of virulence in fungi are located in regions of the genome rich in repetitive elements and transposons (Howlett *et al.*, 2015; Meile *et al.*, 2020). As an example, all genes encoding effector production in *Leptosphaeria maculans* are located in TA-rich regions, consisting largely of degenerate transposable elements, suggesting that these areas may evolve more rapidly than other parts of the genome (Li; Cornelissen; Rep, 2020).

Avirulence proteins function as effectors, a designation given to small proteins whose function is to facilitate infection or induce an immune response in the host plant (Howlett *et al.*, 2015). These proteins are produced by fungi and, when in contact with the host, activate the recognition system by resistance proteins (R proteins) in the plant. This activates the plant's immune system, resulting in resistance to infection. These avirulence factors are

diverse, and are regulated by different alleles in fungal populations, and such genetic variability allows pathogens to adapt quickly to plant defenses. Pathogens can mutate in their avirulence genes, leading to loss of recognition by R proteins in plants. This can occur through deletions, non-synonymous mutations, or loss of expression, allowing the pathogen to evade the plant's immune response and consequently become virulent (Li; Cornelissen; Rep, 2020). This can be observed in the case of the breakdown of canola's resistance to the pathogenic fungus *Leptosphaeria maculans* in Australia. The research conducted by Howlett *et al.* (2015) identified that the breakdown of resistance was related to polymorphisms in the avirulence genes, called AvrLm1 and AvrLm6. These genes are located in regions of the *L. maculans* genome that are rich in transposons and have low recombination, a genomic structure that facilitates mutation, allowing the avirulence genes to be rapidly altered or even deleted, resulting in strains of the fungus that can infect canola cultivars that previously showed resistance (Howlett *et al.*, 2015).

In addition to transposable elements, another characteristic of sites where effector genes are found in the genome is the presence of heterochromatin, which means that the implications of epigenetics for the development of virulence in fungi are significant and multifaceted. The study by Meile *et al.* (2020) with the pathogenic fungus *Zymoseptoria tritici* showed that effector genes located in heterochromatic regions of the genome were associated with repressive epigenetic modifications in conditions of hostlessness, such as the trimethylation of lysine 27 of histone H3. However, upon contact with the host, chromatin decondensation was induced, accompanied by a reduction in histone repressive modifications. This change correlates with the induction of effector genes during the initial stage of infection (Meile *et al.*, 2020).

Transcription factors (TFs) are proteins that bind to specific DNA sequences and regulate the transcription of genes, so they play crucial roles in regulating the expression of virulence-related genes in phytopathogenic fungi, after all they regulate the expression of genes that contribute to pathogenicity and virulence. The annotation of fungal genomes provided the identification and functional characterization of several families of transcription factors. The largest class of transcription factors in fungi is those coordinated by zinc, called "zinc fingers", whose main families are Zn2Cys6, C2H2, GATA, bZIP, bHLH, HD/Hox, Velvet and Gti1/Pac2 (John *et al.*, 2021).

The FT "zinc fingers" are associated with genes that encode a wide range of functions that interfere with the virulence capacity of fungi, from toxin production to growth, development, and adaptation functions. FT Mft4, for example, regulates the formation of appressorium in the species *Colletotrichum orbiculare*, a mechanical invasion structure

produced by fungi that facilitates their entry into host tissues. FT EBR1 is associated with the development and branching of hyphae. Mutants with absence of this TF have impaired infection capacity, since the formation of hyphae in the extremities becomes deficient (John *et al.*, 2021). Some fungi such as *Fusarium graminearum* have accessory chromosomes, containing genes and FTs that can also affect virulence in these species, as is the case of FTs Ftf1 and Ftf2 in *Fusarium oxysporum*. These are associated with the production of a large number of virulence factors, such as the so-called SIX (Secreted-in-Xylem) effectors, and the expansion of Ftf1 gene expression is positively related to virulence. However, this phenotype is expressed only in the presence of the Ftf1 gene and its orthologs located on accessory chromosomes, which can be acquired by horizontal gene transfer or by duplication and neofunctionalization, making a non-pathogenic strain virulent (John *et al.*, 2021).

7 FUNGI-HOST INTERACTION

According to the authors Peixoto-Neto *et al.* (2022), plants establish themselves in their habitats and interact with various associations or species of organisms. Among these associations, endophytic microorganisms are the target of greater attention due to their complex interaction with various plant cultures. Endophytic fungi are a rich source of new natural products and biological properties. They are practically positioned in an unexplored ecological habitat and have secondary metabolites that are particularly effective and interesting, probably due to their metabolic interactions during the host's life (Kusari *et al.*, 2012).

In fact, from endophytic fungi, the natural products produced have a wide variety of biological activities, highlighting their antimicrobial, antioxidant (Jalgaonwala *et al.*, 2011), immunosuppressive, and antiviral properties (Strobel; Daisy, 2003) and cytotoxic (Aly *et al.*, 2011). According to Araújo *et al.* (2010), endophytic microorganisms occupy an ecological position analogous to that of phytopathogens and can, therefore, control them through competition between nutrients, producing antagonistic stimuli, causing parasitism in the pathogen or even inducing the plant to acquire resistance. The synthesis of compounds, such as antibiotics, suggests that endophytes can control plant diseases.

According to studies carried out by Gunatilaka (2006), the association between an endophyte and its host depends on the variation of each host, and its interactions remain poorly known. Esposito and Azevedo (2010) also state that this association allows us to suppose that there was a coevolution between these microorganisms and their hosts, thus characterizing an intimate mutualistic relationship, in which the endophytes received their nutrients and protection and the plant itself presented benefits resulting from such interaction,

such as a greater capacity for resistance in environments with intense stress caused by the joint action of biotic factors (insects, herbs, parasitic nematodes and phytopathogenic microorganisms) as well as abiotic factors (pH, temperature, water stress, strong winds, salinity, etc.).

A wide variety of endophytic microorganisms are present internally in plants where the composition can vary according to the host, geographic distribution, plant age, and environmental and seasonal conditions, such as altitude and rainfall (Jalgaonwala *et al.*, 2011, Santos *et al.*, 2013). The beneficial relationship of the interaction between fungus and host can be characterized in the action of protection, feeding and stimulus production by the fungus, which sufficiently increases vegetative development, reproduction and the resistance capacity of plants in the environment (Santos *et al.*, 2013).

Kusari *et al.* (2012), described the chemical interactions as complex between microorganisms and their hosts, emphasizing the existence of numerous chemical and physical barriers to the successful establishment of an association, which must be overcome. The possibility of balanced antagonism is determined by the principle that when an endophyte succeeds in preventing the mobilization of its host's defense mechanisms, ensuring its own capacity for resistance, i.e. its survival, before being neutralized by toxic metabolites of the host, it manages to develop within its host without causing visible manifestations at the level of infection in the event of balanced antagonism between the crop and the host. The association between the virulence of the fungus and the defense capacity of the plant remains apparently asymptomatic and avirulent, so that both are totally balanced. If the mechanisms responsible in the plant for its defense are opposed to the determinants of the virulence of the fungus, it will die. On the other hand, if the plant submits to the virulence of the fungus, this relationship between plant and pathogen will cause the appearance of a disease in the plant. The possibility that many endophytes are silent pathogens implies the ability to be conditioned by certain intrinsic or environmental circumstances to express certain parameters that can lead to pathogenicity.

8 DIAGNOSIS AND DETECTION OF VIRULENT FUNGI

It is critical to identify pathogens in plants before disease symptoms manifest in order to track plant health and establish an efficient informed disease management (IDM) strategy. As several fungal pathogens cause similar changes in plants during the evolution of the disease, it is crucial to distinguish the causative species.

The conventional methods used for the detection of virulent fungi in plants depend on the interpretation of visual symptoms and/or the isolation, culture and laboratory identification

of the pathogen (Dyussebayev *et al.*, 2021). The accuracy and reliability of these methods depend on the experience and skill of the researcher. In addition, when rapid results are needed, diagnosis requiring culture may be ineffective (Mccartney *et al.*, 2003; Dyussebayev *et al.*, 2021).

With the advancement of research, newer methods are increasingly being applied to the diagnosis of plant pathogens, presenting potential advantages over conventional diagnostic methods because they are safer and faster. We can mention the methods based on polymerase chain reaction (PCR), identification of emerging pathogens by next-generation sequencing (NGS), detection by mass spectrometry (MALDI-TOF-MS) and loop-mediated isothermal amplification (LAMP) (Mccartney *et al.*, 2003; Malarczyk; Panek; Frac, 2019).

8.1 PCR-BASED TECHNIQUES

The advent of polymerase chain reaction (PCR)-based assays has revolutionized accurate identification of various plant pathogens in disease management, including fungi (Hariharan; Prasannath, 2021). In this technique, a piece of DNA template is amplified exponentially through several cycles of denaturation, extension, final extension, girdling, and final retention reactions at different temperatures using specific primers, deoxyribonucleotide triphosphates (dNTPs), and a thermostable Taq DNA polymerase in buffer solution (Malarczyk; Panek; Frac, 2019; Hariharan; Prasannath, 2021).

The end-point PCR method aims to design specific oligonucleotides that target certain fungal species or universal primers to amplify multiple pathogens followed by sequencing. The identity of each isolated fungus can be determined by comparison with ex-type cultures available in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST) analysis (Mccartney *et al.*, 2003). *Cannabis sativa* is increasingly being cultivated in Canada for medicinal purposes. This crop suffers from the incidence of powdery mildew, whose symptoms are white circular spots consisting of epiphytic mycelia and conidia on the leaf surface. To confirm the identity of the pathogen in Canada, the inner transcribed spacer region (ITS) of the rDNA was amplified by PCR with endpoint primers with ITS1-f and ITS4 primers and sequenced (Pépin; Punja; Joly *et al.*, 2018).

Nested PCR is a modified version of end-point PCR that uses two sets of primer pairs, one external and one internal, directed at two rounds of PCR amplification to increase specificity and sensitivity in order to generate early diagnosis of pathogens (Hariharan; Prasannath, 2021). Greater yam disease caused by *Colletotrichum gloeosporioides* (Raj *et al.*, 2013) and eucalyptus dieback disease caused by *Cylindrocladium scoparium* were also detected by this technique (Qiao *et al.*, 2016).

The multiplex PCR assay uses a reaction mixture with multiple primer pairs that allows simultaneous amplification of multiple pathogens. The generated amplicons can then be separated and visualized using electrophoresis (Mccartney *et al.*, 2003). In a study using multiplex PCR, Zhenyan *et al.* (2021) compared the internal transcribed spacer regions of the ribosomal DNA sequences of four widely disseminated pathogens in *Dendrobium officinale* (*Sclerotium delphinii*, *Colletotrichum gloeosporioides*, *Alternaria alternata*, and *Cladosporium cladosporioides*). The sensitivity for *S. delphinii*, *C. cladosporioides* and *A. alternata* was 10 fg/μL and 10 pg/μL for *C. gloeosporioides* (Zhenyan *et al.*, 2021).

Quantitative PCR (qPCR) allows for the detection and quantification of specific DNA or RNA sequences of plant pathogenic fungi in a real-time PCR reaction mix. This technique allows the duplication of DNA strands by measuring the fluorescence emitted in each cycle. The intensity of the fluorescence indicates the amount of DNA in the sample (Kumar *et al.*, 2020). An emerging and hypervirulent plant fungal pathogen, *Cryphonectria parasitica* causes several diseases (late blight, lethal bark cankers, wilt, and regressive death) in chestnut trees, *Castanea dentata*, and *C. sativa*. Through molecular diagnosis by qPCR, it was possible to detect the fungus *C. parasitica* using ITS rDNA sequences with a sensitivity of 2 fg of genomic DNA, which was equivalent to the single spore of a pathogen (Chandelier *et al.*, 2019).

The BIO-PCR assay is a modification of the end-point PCR technique that involves a pre-assay incubation step in a diseased sample to increase pathogen biomass and has been effectively used to detect seed-borne fungal pathogens (Kumar *et al.*, 2020). Pecchia *et al.* (2019) diagnosed an airborne disease caused by *Colletotrichum lupini* through this method. The researchers used Yeast Malt Broth to enrich the biomass of *C. lupini*. Thus, a pair of primers was developed based on the IGS rDNA sequence for the detection of this pathogen (Pecchia *et al.*, 2019).

Magnetic capture hybridization PCR (MCH-PCR) utilizes DNA isolation with a purification phase that contains hybridization with single-stranded DNA probe (ssDNA) in magnetic beads followed by PCR amplification of target DNA sequences (Hariharan; Prasannath, 2021). MCH-PCR has been shown to be effective in identifying pathogenic microorganisms in materials that contain compounds that inhibit CRP, such as *Botrytis aclada* present in onion seeds (Mancini; Murolo; Romanazzi, 2016).

8.2 NGS

The main steps involved in next-generation sequencing (NGS) consist of DNA isolation and fragmentation, library preparation, massive parallel sequencing, bioinformatics analysis, and annotation and interpretation of variants/mutations (Qin, 2019). NGS has been used to

characterize the pathogen of other crops, such as oak, ginseng, tomato, strawberry, potato, and banana. Similarly, the metabarcoding approach is widely used to study oomycetes, especially *Phytophthora* spp (Aragona et al., 2022).

8.3 MALDI-TOF-MS

The matrix-assisted laser desorption/ionization technique – time-of-flight mass spectrometry (MALDI TOF-MS) is a technique that compares cell spectra to a database to identify microorganisms. This methodology relies on the detection of the mass-to-charge ratio (m/z) of ribosomal proteins, which helps to provide a unique mass spectrum of microorganisms in a short period of time (Ashfaq; Da'na; Al-Ghouti, 2022). The authors Chun; Gopal; Muthu (2022) investigated the secretome of biocontrol agents, such as *Bacillus* strains EU07, QST713, and FZB24 that control the fungal pathogen causing wilt *Fusarium oxysporum*, using LC ESI MS MS/MS and gel electrophoresis 1 DE, gel digestion, and MALDI-TOF MS. Revealing that the presence of enzymes such as cellulases, proteases, 1,4- β -glucanase, and other hydrolases can degrade the fungal cell wall (Chun; Gopal; Muthu, 2022).

8.4 LAMP

The loop-mediated amplification (LAMP) technique has become a relevant diagnostic tool in plant disease diagnostics and has enormous potential in plant disease management. The reaction has two main phases: an initial phase of cyclic amplification, followed by a stretching phase (Panno *et al.*, 2020). In a LAMP test, two inner primers, the inner forward primer (FIP) and the inner primer backward (BIP), as well as a loop-backward primer (B-Loop), are employed to identify six unique sequences in the nucleic acid (Panno *et al.*, 2020; Hariharan; Prasannath, 2021). *Uromyces betae*, the fungus responsible for beet rust, was detected in 30 minutes using the LAMP test, focused on the cytochrome b DNA sequence (Kaczmarek *et al.*, 2019). A disseminated pathogen, *Sclerotinia sclerotiorum*, present in a wide variety of hosts, including rapeseed, was identified through an Sso5-focused LAMP test and a blue hydroxynaphthol (HNB) marker for its identification. The LAMP technique limited the identification of *S. sclerotiorum* to $0.1 \text{ fg } \mu\text{l}^{-1}$ of genomic DNA per reaction, a significantly lower value ($100 \text{ fg } \mu\text{l}^{-1}$) than the end-point PCR test (Duan *et al.*, 2014).

9 PREVENTION AND CONTROL STRATEGIES

The best strategy for fungal control is to prevent the entry of the pathogen into the cultivation area and adopt appropriate crop management to coexist with the pathogen in

areas already affected. In addition, the planting of resistant cultivars is essential (Costa *et al.*, 2015; Mostert *et al.*, 2017).

However, these measures are not always sufficient, making the use of chemical pesticides necessary to ensure agricultural productivity and quality (Kimati, 1995). However, a growing alternative is Biological Control, carried out by means of microorganisms, which has shown good results in reducing dependence on agrochemicals (Morandi; Bettiol, 2009).

A challenge for the use of mycoviruses in biological control is the vegetative incompatibility of fungi. To overcome it, strategies such as the use of chemical compounds that suppress programmed cell death (PCD) mechanisms are proposed (Xie; Jiang, 2014), including substances that sequester reactive oxygen (Hutchison *et al.*, 2005) and zinc compounds that inhibit enzymes associated with apoptosis and promote hyphal fusion (Ikeda *et al.*, 2013). In addition, the selection of mycoviruses capable of suppressing gene silencing may facilitate the introduction of hypovirulence-inducing mycoviruses (Xie & Jiang, 2014). Research on potential mycovirus vectors, such as infected mycoparasites, nematodes, and mycophagous insects, can also contribute to advances in this strategy (Xie & Jiang, 2014).

10 CONCLUSION

The mechanisms of virulence in fungi represent a complex evolutionary arsenal that allows these organisms to colonize, infect and survive in plant hosts, causing significant impacts on agriculture and food security. This study highlighted the diversity of strategies adopted by phytopathogenic fungi, from the initial adhesion mediated by cell wall components and biofilm formation to the production of specific and non-specific toxins, capable of subverting physiological processes of plants. The evasion capacity of the plant immune system, whether by structural modification of chitin, secretion of effectors or manipulation of hormonal pathways, reinforces the sophistication of these pathogens in establishing persistent infections.

The genetic regulation of virulence, mediated by transcription factors and dynamic gene clusters, combined with metabolic plasticity, evidences the rapid adaptation of fungi to selective pressures, such as the use of fungicides. In this context, the emergence of antifungal resistance and the persistence of soil survival structures highlight the need for integrated management strategies. Advanced diagnostic methods, such as quantitative PCR, next-generation sequencing (NGS), and isothermal amplification techniques (LAMP), have emerged as promising tools for early detection and monitoring of pathogens.

However, effective control of these diseases requires multifaceted approaches. The development of resistant cultivars, the application of biocontrol agents and the modulation of

signaling molecules, such as farnesol in quorum sensing, point to sustainable alternatives that reduce dependence on chemicals. Future research should explore the interaction between epigenetic factors, fungal effector diversity, and plant defense mechanisms, as well as integrate omics technologies to deviate host-pathogen signaling networks.

In summary, understanding the complexity of fungal virulence mechanisms not only expands basic knowledge about ecological interactions, but also provides subsidies for innovations in disease management. The adoption of resilient agricultural practices and investment in molecular biology-based solutions are essential to mitigate economic losses and ensure the sustainability of agri-food systems in the face of a scenario of climate change and increasing pathogenic pressures.

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