CHAPTER

5

Chemical communication between *Trichoderma* and plants

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Introduction

Trichoderma is a diverse genus of ascomycete fungi, with over 250 recognized species (Bissett et al., 2015). The genomes of 23 isolates distributed in 11 species have been fully sequenced and are publicly available in diverse sources (https://genome.jgi.doe.gov/mycocosm/home/releases?flt=trichoderma). The group has a wide variety of habitats and similarly diverse life histories. They are found in soils worldwide, and also function as opportunistic avirulent plant symbionts (Harman et al., 2004). The ability of *Trichoderma* spp. to thrive in such a wide range of habitats is linked to their ability to produce a number of bioactive molecules, such as lytic enzymes and antibiotics (Woo et al., 2006). This has made them popular for use in the industrial biosynthesis of compounds such as cellulases, xylanases, and chitinases (Horta et al., 2018; Fang et al., 2019). *Trichoderma* spp. are frequently found in symbiotic relationships with plants and are capable of increasing plant tolerance to a wide range of abiotic and biotic stresses. This makes them popular as biocontrol agents (BCAs) in agriculture (Kubicek et al., 2011). Collectively, these attributes make *Trichoderma* spp. great candidates for studies of plant−microbe interactions (Druzhinina et al., 2011; Hermosa et al., 2013; Alonso-Ramirez et al., 2014; Lee et al., 2015; Morán-Diez et al., 2015; Garnica-Vergara et al., 2016; Saravanakumar et al., 2016; Schmoll et al., 2016; Coppola et al., 2017; Guzmán-Guzmán et al., 2017; Martínez-Medina et al., 2017; Nieto-Jacobo et al., 2017; Mendoza-Mendoza et al., 2018; Mukherjee et al., 2018; Nogueira-Lopez et al., 2018).

Trichoderma spp. establish symbiotic relationships with many plant species, including agriculturally relevant species such as maize and tomato (Chacon et al., 2007; Sobowale et al., 2007; Degani et al., 2013; Morán-Diez et al., 2015; Saravanakumar et al., 2016; Coppola et al., 2017; Nogueira-Lopez et al., 2018). Here, penetration into plant root tissue is assumed to occur via a penetration peg to punch through cell walls (Yedidia et al., 1999; Nogueira-Lopez et al., 2018). As the fungus enters the plant roots, the plant deposits callose, limiting the extent of fungal growth (Ellinger et al., 2013; Alonso-Ramirez et al., 2014; Nawrocka et al., 2018). Interactions inside the plant cell are not well studied, but several other fungi form haustoria, which allow uptake of nutrients from the cell, as well as chemical signaling across the extrahaustorial membrane (Catanzariti et al., 2007). Once settled within the plant, the fungus has a wide range of beneficial effects upon its host: root growth, nutrient uptake, and plant growth are all significantly enhanced by *Trichoderma* colonization (Harman et al., 2004; Hermosa et al., 2013). Perhaps most significantly, plant tolerance to pathogenic attack is increased. This occurs via an enhanced immune response predominantly by inducing the plant immune system, but also by mycoparasitism and antibiosis (Shoresh et al., 2010).

Trichoderma induces plant defense via a number of mechanisms. The small-secreted protein, Sm1 (and homologous proteins Epl1 and Sm2), a signaling protein related to pathogenic phytotoxic proteins, was discovered to be secreted by both *Trichoderma atroviride* and *Trichoderma virens* (Djonovic et al., 2007; Crutcher et al., 2015; Gaderer et al., 2015; Salas-Marina et al., 2015). This protein is not toxic to the plant, and instead induces a systematic plant immune response. Deletion of the Epl1 gene in *T. atroviride* reduced the protective effect in tomato plants (Salas-Marina et al., 2015). Terpenoids, peptaibols, and other secondary metabolites (SMs) synthesized by *Trichoderma* have also been shown to induce plant immune responses (Vinale et al., 2008a,b; Shah, 2009; Shoresh et al., 2010). This results in a heightened response to pathogenic attack via priming of induced systemic resistance (ISR) and is less costly to the plant than a constitutive expression of resistance genes.

Trichoderma spp. are highly studied worldwide because of their properties as BCAs. The major mechanisms that *Trichoderma* utilize for controlling plant pathogens are direct competition for same niche-nutrients, mycoparasitism, production of cell wall–degrading enzymes (CWDEs: cellulases, chitinases, and glucanases), and SMs, such as antibiotics. SMs can have a dual function and either induce systemic resistance in plants (Brotman et al., 2010) or restrict the growth of potential microbial competitors. Once established in the plant, *Trichoderma* spp. also act to promote plant growth, increase nutrient availability, improve crop production, and enhance disease resistance (Vinale et al., 2008a,b; Mukherjee et al., 2013), further favoring their utility in agriculture.

Several members of the genus are able to parasitize fungal and nematode plant pathogens (Harman, 2006; Samuels, 2006; Chaverri and Samuels, 2013). In this instance, *Trichoderma* colonizes the root epidermis and cortical cells releasing bioactive molecules that cause transcriptional and proteomic changes in the plant host for at least the life of the annual crop (Lu et al., 2004; Harman, 2006; Vinale et al., 2008a,b). Because of this interaction, plant resistance pathways are induced, as well as plant growth and nutrient uptake. Furthermore, *Trichoderma* spp. have been reported to induce the systemic and localized resistance in plants (Djonovic et al., 2006, 2007; Hoitink et al., 2006; Shoresh et al., 2006).

Trichoderma can provide remarkable benefits to plants. Indeed, successful colonization enhances nutrient uptake, promotes the solubilization of soil nutrients, and enhances root development by increasing the number of root hairs and promoting deeper rooting (Harman, 2006; Chaverri and Samuels, 2013; López-Bucio et al., 2015). Thus, *Trichoderma* has been successfully used in both horticulture (fruits, vegetables, and flowers across many different cultivars) and plant agriculture to obtain higher yield and for the suppression of soil pathogens, promotion of plant growth and abiotic stress resistance (Harman, 2006; Brotman et al., 2013; López-Bucio et al., 2015; Zhang et al., 2016).

Endophytic *Trichoderma*

Plants and endophytic microbes have coevolved over millions of years; consequently, they have created a mutualistic symbiosis where both organisms obtain benefits from this interaction. Plant tissues are microecosystems where a variety of microorganism taxa interact with each other to create a biofunctional microenvironment. Endophytic mutualistic fungi differ from plant-pathogenic endophytes in that they must maintain a stable relationship with their host. Although these fungi are beneficial to the host, it has recently been shown that they also elicit an immune response, in a manner similar to that of pathogens (Lo Presti et al., 2015). This immune response does not, however, escalate to the same levels as those involved in pathogen clearing. The plants response to mutualistic fungi is regulated by a complex communication system established between the plant and fungus. The exact mechanisms of this communication are unknown; however, signaling networks involving jasmonic acid, ethylene, and salicylic acid are thought to be involved (Van Wees et al., 2008). These signaling networks are all part of systematic defense signaling pathways that are triggered in response to the pathogenic attack (Shah, 2009).

Endophytes must manipulate plant-signaling networks to adjust the plant immune response. Plant defense proteins, such as NPR1 and MYB72, are involved in communicating with beneficial microbes and are also known to be part of defense signaling in response to

pathogens (Van Wees et al., 2008; Rafiqi et al., 2013). Several studies have shown that single gene alterations can seriously alter the interactions of both pathogens and endophytes; pathogenic species were rendered harmless, and endophytic species were made virulent, by addition or modification of single genes (Eaton et al., 2011; Charlton et al., 2012; Kuo et al., 2014; Zhao et al., 2014). Particularly important is the ability of some fungi to switch lifestyle based on environmental or physiological conditions (Kuo et al., 2014). It is clear that closely related pathways that respond to both pathogens and endophytes exist. These pathways are subject to extremely tight regulation.

Bacteria and fungi endophytes inhabit diverse plant species, colonizing the internal tissues of the roots, pseudostem, and leaves of living plants in all or part of their life cycle (Rodriguez et al., 2009; Santoyo et al., 2016; Brader et al., 2017). The main entry point of endophytic microorganisms is through the rhizosphere (Backman and Sikora, 2008), followed by the phyllosphere (Hardoim et al., 2015). The rhizosphere is defined as the zone around the root where plants produce root exudates with high amounts of carbohydrates, organic acids, and amino acids attracting diverse microorganisms by quimiotaxis (Bais et al., 2006). Before attempting to penetrate the host plant, soil microbiomes struggle and compete for nutrients within the rhizosphere, in order to establish a successful infection. This is when plant-nonpathogenic endophytes play their first main role, outcompeting other microorganisms (plant-pathogenic endophytes) in order to enter and colonize the plant (Brader et al., 2017), thus establishing a mutualistic symbiosis that directly influences plant health (Berendsen et al., 2012). Indeed, plant endophytes are characterized by conferring beneficial attributes during their mutualistic interactions with plants, such as growth promotion, enhanced disease resistance, facilitation of nutrient acquisition, increased abiotic stress tolerance, and improvements in plant fitness (Schulz and Boyle, 2006; Busby et al., 2016).

A large number of *Trichoderma* species behave as opportunistic-endophytic plant symbionts and have the capacity to penetrate plant tissues in order to create an endophyte−plant beneficial interaction. They act as free-living organisms and are found principally in plant root ecosystems (Harman et al., 2004; Vinale et al., 2008a,b). During this interaction, *Trichoderma* produces compounds that cause substantial changes in the plant architecture, enabling successful colonization. Endophytic *Trichoderma* penetrates the first or second layer of plant root systems, colonizing first the root epidermis and then into the cortex; however, they do not reach the xylem or phloem systems (Chacon et al., 2007). Because of fungal penetration, the plant responds by depositing lignin and callose in the neighborhood cells, permitting superficial root colonization. Observations at the early colonization stage of tomato roots by *Trichoderma harzianum* show the capacity of the fungus to colonize the intercellular spaces without causing cell damage; nevertheless, 48 h postinoculation, some tomato root cells had been colonized intracellularly (Chacon et al., 2007).

It has been proposed that, before colonization, *Trichoderma* establish a chemical dialogue with the plant. During the interaction, *Trichoderma* express and release a cocktail of microbe-associated molecular patterns (MAMPs), damage-associated molecular patterns (DAMPs), and protein-like effectors that can be recognized by plant receptors, inducing beneficial responses in the host and minimizing the stimulation of the plant immune system (Hermosa et al., 2013; Schmoll et al., 2016; Mendoza-Mendoza et al., 2018). Thus, once *Trichoderma* hyphae penetrate the roots, a series of cross-talk molecules likely come into play (Druzhinina et al., 2011; Nogueira-Lopez et al., 2018).

Many metabolites secreted by *Trichoderma* trigger the immune system of the plant. For example, the SMs produced by two different species, *T. harzianum* and *T. atroviride*, induce the upregulation of gene expression of defense-related proteins such as chitinase class IV, endochitinase, and PR-1 (Vinale et al., 2008a,b). In addition, the SM 6-pentyl-alpha-pyrone produced by *T. atroviride* acts as an auxin-like compound inducing plant growth promotion and increasing leaf and root system volume (Vinale et al., 2008a,b). It is likely that each metabolite plays a particular role during the interaction. Furthermore, *Trichoderma* interaction with plant host roots increases sensitivity, allowing the plant to respond more successfully to later pathogen invasion via ISR (Shoresh and Harman, 2008; Saravanakumar et al., 2016) (Fig. 5.1).

Plant defense mechanisms

Induced resistance (IR) is the main mechanism plants use to cope with the threats they encounter during their life cycle. IR is a state where the plant's defensive capacity is enhanced by the appropriate stimuli (Choudhary et al., 2007). The time it takes for a plant to display these defense responses can be the difference between surviving and perishing under the threat of biotic or abiotic stresses such as drought, salinity, herbivory, pathogenic attacks by fungi, insects, parasitic plants, nematodes or viruses, etc. Thus, plants naturally respond to any disruption by activating this mechanism (Heil and Bostock, 2002; Choudhary et al., 2007).

ISR and systemic acquired resistance (SAR) are the two forms of plant IR described until now; however, these do not exclude the existence of alternative mechanisms of defense. These two forms of IR are activated as a result of prior infection or treatment, and subsequently protect the plant to varying degrees against challenges from biotic or abiotic factors (Heil and Bostock, 2002). ISR and SAR are differentiated by the elicitor and regulatory pathway involved in triggering the defense mechanism. SAR induction requires the accumulation of pathogenesis-related (PR) proteins (glucanase and chitinase) and salicylic acid and is a characteristic response against pathogens (Van Loon and Van Strien, 1999; Shoresh et al., 2010), while ISR relies on the jasmonate and ethylene pathways and has been strongly associated with plant growth promoter rhizobacteria/fungi interaction (Choudhary et al., 2007; Shoresh et al., 2010). Interestingly, the ability of *T. virens* to control seedling disease in cotton caused by *Rhizoctonia solani* is not related to antibiotics or mycoparasitism activities, but to the induction of terpenoid phytoalexins in the plant host (Howell et al., 2000), suggesting that ISR activation by *Trichoderma* may represent an important mechanism to control diseases in systemic tissues (Shoresh et al., 2010). This observation has been extended to other *Trichoderma* species and is not restricted to biotic stress protection, but also abiotic stress tolerance (Shoresh et al., 2010). Unfortunately, the signals and the mechanism by which they are transmitted from the roots to the systemic tissues remain unclear, although recent findings suggest that, with *T. atroviride* infection, tomato plants are protected against the root knot nematode, *Meloidogyne incognita*, by a mechanism that involves shifting from a salicylic acid pathway to a jasmonic acid pathway (Martinez-Medina et al., 2017). More interesting, however, is the fact that this protection seems to be transferred between generations (Medeiros et al., 2017).

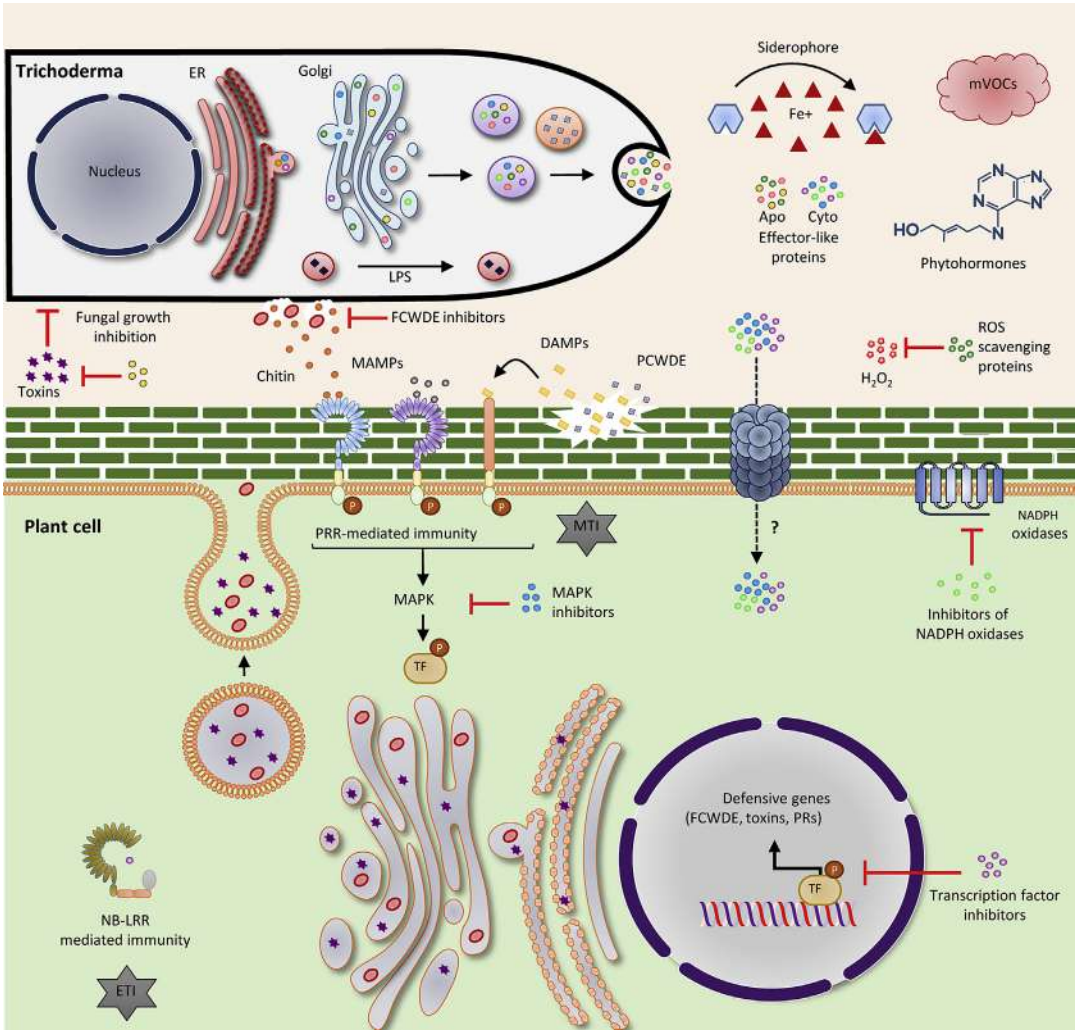

FIGURE 5.1 **Molecular Signaling Microbe–Plant Interaction. Fungi Continuously Release Proteins and Secondary Metabolites to Interact With Their Host Plant Cell.** Firstly, microbe-associated molecular patterns (MAMPs), e.g., chitin, or damage-associated molecular patterns (DAMPs), e.g., plant cell wall oligosaccharides, are detected by pattern recognition receptors (PRRs) activating MAMP-triggered immunity (MTI). Secondly, fungi deliver apoplastic and cytoplasmic effector-like proteins to block at different levels the MTI response, but these proteins could be recognized by nucleotide binding and leucine-rich repeat (NB-LRR) immune receptors that trigger the second layer of defense called effector-triggered immunity (ETI). Finally, secretion of fungal secondary metabolites, e.g., siderophore, phytohormones, and microbial volatile organic compounds (mVOCs) that function as messengers between fungi and plants, has a direct impact in plant nutrition, defense, and signaling. Abbreviations: *ER*, endoplasmic reticulum; *FCWDEs*, fungal cell wall–degrading enzymes; *Fe*, iron; H_2O_2, hydrogen peroxide; *LPS*, leaderless protein secretion; *MAPK*, mitogen-activated protein kinase; *NADPH*, nicotinamide adenine dinucleotide phosphate; *P*, phosphorus; *PCWDEs*, plant cell wall–degrading enzymes; *PRs*, pathogenesis-related proteins; *ROS*, reactive oxygen species; *TF*, transcription factor. *Image adapted from Schmoll, M, Dattenböck, C, Carreras-Villaseñor, N, Mendoza-Mendoza, A, Tisch, D, et al., 2016. The genomes of three uneven siblings: footprints of the lifestyles of three* Trichoderma *species. Microbiol. Mol. Biol. Rev. 80, 205–327 with modifications.*

The plant immune system

The plant defense system is composed of a robust, three-layer mechanism consisting of one physical layer and two layers based on molecular pattern recognition that has significant crossover with each other. This system is capable of suppressing the majority of microbial invaders with only extremely well-adapted colonizers capable of causing sustained invasion. The first layer of plant defense consists of a physical barrier that microbes must penetrate. The second layer consists of enzymes and receptors that trigger active immune responses. The third layer is composed of effector-detecting receptors (Jones and Dangl, 2006). Both the second and the third layer have evolved to respond to any infection as part of a conserved innate immune system, which involves plasma membrane–localized and intracellular immune receptors, and are capable of greatly escalating a plant's response to microbes, eventually triggering systemic immunity (Dangl et al., 2013).

The physical barrier that comprises the first layer of plant immunity consists of structures made from strong molecular bonds and cross-linking of complex sugar and fat molecules (Malinovsky et al., 2014). This layer is readily observable, consisting of wax, cuticle layers, cell walls, and stomata, all of which must be overcome to gain physical entry into the plant (Malinovsky et al., 2014).

The molecular second layer provides basal defense and recognizes self from non-self structures. This enables the plant to respond to molecules known as MAMPs or pathogen-associated molecular patterns (PAMPs), e.g., chitin and flagellin (Jones and Dangl, 2006), as well as endogenous elicitors, which are described as DAMPs, e.g., cell wall fragments (Ibrahim et al., 2013). The recognition of DAMPs allows the plant to identify damage caused to itself by microbial invaders or colonizers (generally via lytic enzyme release products, such as sugar molecules) (Benedetti et al., 2015). This second layer leads to rapid and enhanced defense resulting from the recognition of pathogen effectors (virulence factors), either directly or indirectly by their effect on host targets (De Wit et al., 2009; Cook et al., 2015).

A highly conserved form of adaptive immunity forms the third layer of plant defense. In the soil, biotrophic, hemibiotrophic, and necrotrophic microbes are constantly fighting for nutrients and trying to colonize plant roots. After plant cells sense MAMPs/DAMPs via pattern recognition receptors (PRRs) in the second layer of defense, they activate basal MAMP/PAMP-triggered immunity (MTI/PTI), which prevents further penetration attempts of the host epidermal cells (Postel and Kemmerling, 2009; Miller et al., 2017). Nevertheless, successful pathogens and beneficial microorganisms might overcome MTI/PTI by the release of effectors or effector-like proteins. To this microbial response, plants have coevolved a third layer of defense, which is an adaptive effector-triggered immunity (ETI). ETI is activated based on the recognition of effectors via R proteins (Thomma et al., 2011) and is more specific than the MTI/PTI response, generally leading to a hypersensitive response (HR) at the infection site resulting in disease resistance (Boller and Felix, 2009; Cui et al., 2015). Furthermore, receptor-like kinases, receptor-like proteins, and mitogen-activated protein kinase (MAPK) cascades are fundamental signaling modules downstream of PRR activation by MAMPs/DAMPs, triggering biochemical, biophysical, and molecular changes in the plant, for example, reactive oxygen species (ROS) generation, stomatal closure, and defense gene activation, respectively (Meng and Zhang, 2013; Tang et al., 2017).

It has recently been shown that endophytes also elicit an immune response while attempting to colonize their host, even though they are generally beneficial (Van Wees et al., 2008). Endophytic mutualistic fungi maintain a stable relationship with the host; however, the immune response they encounter during colonization appears to share significant similarity to the plant response to pathogenic attack. Indeed, up to 40% of the genes responding to mutualistic fungi may also be involved in defense responses (Van Wees et al., 2008). This suggests that pathogenic models may provide useful and important insights into the mechanisms by which mutualistic endophytes colonize their plant hosts.

Physical barriers to microbe colonization

For a microbe to invade a plant, the physical barriers of the plant must be breached. The plant cell wall is composed of a complex interwoven linkage of cellulose, hemicellulose, pectin, and lignin, all of which are arranged to maximize cross-linking (Malinovsky et al., 2014). This makes a molecular attack upon the cell wall difficult—yet microbes have found a number of ways to do so. Pathogens may evade the cell wall and cuticle entirely by attempting to enter through stomata, as they provide a ready-made entrance to the plant—albeit one protected by guard cells (Arnaud and Hwang, 2015). Guard cells may be overcome by a pathogen ability to initiate stomatal opening (Schulze-Lefert and Robatzek, 2006). Other fungi produce infection structures (appressoria or appressoria-like structures) which adhere to the cell wall. These resemble a hemisphere, with a flattened bottom in contact with the plant cell wall. On the underside of the appressorium, a penetration peg forms, which is then mechanically forced through the plant cell wall. Melanized appressoria can exert sufficient force to breach membranes made of metals such as gold, allowing the fungus easy access to plant cells by penetrating through outer plant root and leaf surfaces (Ryder and Talbot, 2015). Nonmelanised appressoria, on the other hand, often lack the ability to penetrate surfaces. Arbuscular mycorrhizal fungi use similar structures, called hyphopodium, to attach and facilitate entry on plant epidermal cells (Rich et al., 2014). Appressoria formation has been observed in a variety of fungi and has been confirmed on different occasions in *Trichoderma* species (Lawry, 2016; Nogueira-Lopez et al., 2018). Other mechanisms of physical penetration include access through wounds on the plant. Fungi also use molecular methods, particularly CWDEs and cell wall loosening molecules (e.g., expansins, such as swollenin), to penetrate the outer layers of the plant, and possibly derive nutrition in the process (Brotman et al., 2008; Gohre and Robatzek, 2008). In brief, CWDEs target cell wall molecules for degradation, and a complex cocktail of enzymes may allow weakening or destruction of the plant cell wall matrix. This may facilitate mechanical entry, or be a reliable method of penetration on its own.

MAMP- and PAMP-triggered interactions

Once within the intercellular spaces of the plant (the apoplast), a more complex series of molecular interactions occur. Major families of pathogens carry distinct molecular markers, several of which are critical to their survival. Examples of these include chitin or flagellin, both critical cell structure components of fungi and bacteria, respectively (Hückelhoven, 2007). The plant innate MTI system reacts to MAMPs allowing broad-spectrum identification of nonplant tissue. MAMP receptors (ligand-binding receptor kinases) are capable of detecting elicitors and instigating an immune response proportional to the level of MAMPs

detected (Bent and Mackey, 2007). These receptors are known as PRRs, consisting of a pattern-binding domain and a kinase domain allowing downstream signaling (Muthamilarasan and Prasad, 2013). The specific binding of flagellin (an MAMP) to FLS2 protein, an LRR receptor kinase (a type of PRR), is a good example of this (Gómez-Gómez and Boller, 2000). Upon receiving the flagellin-triggered signal from the PRR, several molecules (such as serine and cysteine proteases) are released, causing the breakdown of the MAMPs into component molecules, which are then redetected by secondary signaling molecules, escalating the immune response (Gohre and Robatzek, 2008). A similar response is initiated when damage to the plant is detected via DAMPs. PRR receptors will in this case detect breakdown products of the plant itself, caused by the pathogenic attack (Krol et al., 2010). These primary responses are predominantly characterized by localized reactions to the area under attack, consisting of oxidative bursts, localized necrosis, and release of proteases. The extent of signaling will determine whether the response becomes systemic. This process is tightly regulated as it has significant energy costs to the plant.

Microbes that are adapted to a particular host plant have a counter for their host MAMP defense system. This counter takes the form of small-secreted molecules, known as effectors. Effectors are an incredibly diverse set of proteins, which are currently not well understood. Known effector mechanisms include scavenging of MAMPs, degradation of host proteases, and deregulation of primary and secondary signaling pathways in their host (Lo Presti et al., 2015). This type of interaction tends to lead toward evolutionary arms races, with strong selection pressures for the pathogen to evade host immunity, and for the plant to retain it.

Effector-triggered immunity

Effector molecules used by pathogens to overcome MTI/PTI can also be their downfall, as they may be detected by receptors responsible for ETI. Effector molecules produced by pathogens suppress plant MTI/PTI. Detection of effector molecules initiates an immune response that can lead to SAR. Pathogens release another suite of effectors that attempt to counter ETI; however, these may be also detected by plant ETI. Thus, ETI is the key determinant in the ability of plants to resist colonization by pathogens (and other colonizers such as endophytes), and the effector suite of a pathogen is the major determinant of its ability to colonize the plant. Therefore, these effector molecules face significant evolutionary pressure.

Secretion systems

Protein secretion is an ability that all types of cells possess. It is an efficient and controlled mechanism that involves membrane transportation inside the cytoplasm and is fundamental for the proper functioning of the cell (Coulthurst, 2013). The secretome is regulated by different environmental signals and regulatory factors in which organisms modify and optimize their secretory mechanisms to adapt to the changing environment (McCotter et al., 2016). Eukaryotic cells mainly transport and secrete proteins via a conventional secretion system that involves the endoplasmic reticulum (ER)/Golgi apparatus–dependent pathway, which is facilitated by vesicles (Vitale and Denecke, 1999). These extracellular secreted proteins are characterized by a conserved N-terminal signal peptide and the absence of transmembrane domains or anchor membrane signals (Ma et al., 2010).

This conventional ER−Golgi pathway of secretion is common in both plants and fungi. In *Trichoderma*, for example, *Trichoderma reesei* secretes cellulolytic enzymes through this mechanism (Saloheimo and Pakula, 2012). Plants, in response to environmental shifts, also secrete proteins involved in cell wall metabolism, redox regulation, defense/stress, and signaling via this conventional secretion system (Yadav et al., 2015). In fungi, the small-secreted proteins that follow this conventional pathway form part of the fungal secretome in different fungal phyla, such as Ascomycota, Basidiomycota, Glomeromycota, Microspora, and Zygomycota (Kim et al., 2016).

Unconventional protein secretion systems, which release proteins to the plasma membrane and the extracellular space without entering the ER−Golgi conventional pathway of secretion, have been demonstrated in prokaryotic and some eukaryotic organisms (Ding et al., 2012; Lloubes et al., 2013; Robinson et al., 2016; Rabouille, 2017). In this instance in plants, nonvesicular and vesicular modes of transport are used to deliver leaderless secretory proteins into the apoplast under stress conditions (Robinson et al., 2016; Wang et al., 2018b), while bacterial pathogens have specialized mechanisms to secrete molecules that have specific biological functions in their host. These mechanisms in bacteria can operate both directly, through cell contact with the host via the type III, IV, and VI secretion systems, and indirectly, via the type I, II, and outer membrane vesicles systems, depending on their lifestyle and niche (Jha et al., 2005; Coulthurst, 2013; Lloubes et al., 2013; Costa et al., 2015).

The unconventional secretory pathways that operate in fungi are still poorly understood. A novel secretion system has been identified for the cytoplasmic effectors from *Magnaporthe oryzae*, which indicated their accumulation in the biotrophic interfacial complex by a mechanism independent of the ER (Giraldo et al., 2013). Furthermore, unconventional protein secretion has been also observed in other fungal pathogens including *Ustilago maydis, Phytophthora sojae*, and *Verticillium dahlia* (Djamei et al., 2011; Stock et al., 2012; Liu et al., 2014). One suggested mechanism utilized by both fungi and plants to unconventionally deliver proteins during plant−microbe interactions is through the formation of extracellular vesicles called exosomes (Lo Presti and Kahmann, 2017; Rutter and Innes, 2017). Therefore, this suggests that plants, plant-pathogenic fungi, and nonpathogenic endophytic fungi have all evolved distinct secretory mechanisms to deliver a collection of proteins, either in response to microbial infection (pathogens) or in order to manipulate host cell processes (endophytes).

Secretome-mediated plant−fungal interactions

The plant−fungal secretome represents the set of proteins secreted by both the invader and the host. These proteins can be located either on the plant surface or in the extracellular plant space (the apoplast) under constitutive or induced conditions. The apoplast is the space outside the plasma membrane and includes the plant cell wall and the surrounding intercellular space. It is where several physiological processes take place: for example, water transport, nutrient uptake, growth regulation, and gas exchange (Hoson, 1998; Sattelmacher, 2001; Fatima and Senthil-Kumar, 2015). The apoplast is also considered an interface which mediates the molecular cross-talk between microbes and plants (Doehlemann and Hemetsberger, 2013). Plant microbes and their host plants continuously secrete an arsenal of proteins into the apoplast, either by conventional or unconventional secretion systems (Giraldo et al., 2013; Liu et al., 2014). Originally, this conventional secretion system was considered the only mechanism for the secretion of apoplastic proteins, but there is evidence which suggests that

apoplastic proteins are also delivered into the apoplast by leaderless secretory pathways that constitute, on average, 50% of the plant and fungal secretome (Agrawal et al., 2010; Ding et al., 2012; Girard et al., 2013; Delaunois et al., 2014).

Secreted proteins accomplish different biological functions in both the host and the invader. These functions can include the formation and maintenance of cell wall structure, cell-to-cell interaction, enabling nutrient uptake, sensing the external environment, regulating stress responses, and mediating interactions with other organisms (Alexandersson et al., 2013; Yadav et al., 2015; McCotter et al., 2016). Protein families that are commonly found in the plant secretome include proteases, peptidases, glycoside hydrolases, lipases, peroxidases (POs) , cysteine-rich secretory proteins, and PR proteins (Kwon et al., 2008; Alexandersson et al., 2013; Delaunois et al., 2014), which together mediate or control invasive microbial colonization. The microbes involved primarily secrete hydrolytic enzymes and effector proteins to penetrate into plant tissue and overcome plant immune responses, respectively, but they also secrete other families of proteins to influence adhesion and colonization, such as hydrophobins, which are deposited on the host surfaces (Schmidt and Volker, 2011; Gupta et al., 2015).

Apoplastic proteins are secreted by plant microbes during successful interactions. These typically include plant CWDEs, such as endoglucanases, xylanases, cellulases, and pectinases, which degrade the plant's physical barrier against abiotic and biotic factors (Kubicek et al., 2014). Plants counterattack by secreting proteases and hydrophobic proteins into the apoplast to disrupt the function of microbe-associated proteins and effectively perceive MAMPs, which enables a quick response against intruders and maintenance of the integrity of the cell wall (Fernandez et al., 2012; Jashni et al., 2015). Several defense-related proteins are also induced during plant basal defense responses upon infection into the apoplast, including PR proteins which exhibit direct antimicrobial activities. Among the most recognized families of PR proteins present are β-1,3-glucanases, chitinases, thaumatin-like proteins, proteinase inhibitors, POs, and ribonuclease-like proteins. For example, chitinases degrade the chitin present in fungal cell walls, subsequently generating molecules that act as signals that elicit further defensive mechanisms (van Loon et al., 2006).

Plant symbionts and pathogens have developed specific strategies to promote colonization by secreting effectors to evade MTI. Plant microbes secrete molecules, including effector-like proteins, into the apoplast where they can interact with their molecular targets or are translocated into the plant cell cytoplasm blocking downstream signals, thereby suppressing MTI. Examples of conventional secreted effectors have been reported in both symbiotic and pathogenic microorganisms: the PIIN_08944 effector contributes to root colonization in *Piriformospora indica* (Akum et al., 2015); the LysM effector Ecp6 prevents elicitation of host immunity during infection by sequestering chitin oligosaccharides of the fungus *Cladosporium fulvum* (de Jonge et al., 2010); and toxin-like protein, ToxB, is secreted into the apoplast by the necrotrophic fungus *Pyrenophora tritici-repentis* and is necessary for complete disease development in wheat (Figueroa et al., 2015).

Unconventionally secreted effector proteins also play important roles in the manipulation of plant defense processes. For example, the protein chorismate mutase, Cmu1, secreted by *U. maydis*, manipulates the metabolome of neighboring cells to favor parasite infection (Djamei et al., 2011). Other examples of unconventionally secreted effectors are Pslsc1 and Vdlsc1, from *P. sojae* and *V. dahlia*, respectively, both of which participate in the suppression

of the SA pathway to alter plant immune responses (Liu et al., 2014). The direct or indirect recognition of effectors by plant receptors activates ETI that is stronger than MTI, but both MTI and ETI are recognized as the principal plant immune responses against microbes (Stotz et al., 2014).

Proteomic tools, such as mass spectrometry, have enabled the identification of proteins of the secretome during complex physiological cell processes, such as microbe–host interactions (Schmidt and Volker, 2011; Delaunois et al., 2014; Gupta et al., 2015). For example, in the study of the apoplastic secretome of rice plants colonized by *M. oryzae*, more than 200 proteins were found to be secreted into the apoplast, of which several may act as effector proteins (Kim et al., 2013). In the case of endophytic *Trichoderma* species, which have the capacity to penetrate root tissues and subsequently colonize the host plant, recent studies have focused on the secretome of *T. virens* in the presence of maize roots (Lamdan et al., 2015; Nogueira-Lopez et al., 2018). *T. virens* guides the secretome of maize by reducing the PO activity in the apoplast as a possible strategy to manipulate plant immune responses (Nogueira-Lopez et al., 2018). This most likely mediated by the secretion of *Trichoderma* effector proteins during roots interaction (Lamdan et al., 2015; Schmoll et al., 2016; Guzmán-Guzmán et al., 2017; Mendoza-Mendoza et al., 2018; Nogueira-Lopez et al., 2018).

Future research enabling the role of the potential effectors released in the apoplast may reveal interesting findings in this unique nonpathogenic invader–host communication.

Molecular interplay during fungal–plant interactions

Role of hydrophobins in plant recognition and attachment

During their life cycle, filamentous fungi produce small cysteine-rich surface-active amphipathic proteins called hydrophobins. The main roles of hydrophobins are as structural components for fungal growth and mediation of the cell with the environment (Linder et al., 2005; Wu et al., 2017; Appels et al., 2018). Hydrophobins are divided into two classes (I and II), based on their solubility and hydropathy characteristics, and the type of layer they form. During the fungal–plant interaction, fungal hydrophobins are produced in the first stages of root sensing. Their biological functions are the recognition and adhesion to host surfaces where they positively influence root colonization ability (Viterbo and Chet, 2006; Dubey et al., 2014; Moonjely et al., 2018). In addition, it has been hypothesized that hydrophobins might function as protection from detection by the plant (Viterbo and Chet, 2006; Degani et al., 2013), and act as elicitors of the plant defense response (Plett and Martin, 2011; Ruocco et al., 2015). When the symbiotic ability in the ericoid mycorrhizal fungus *Oidiodendron maius* was tested, it was found that the hydrophobin-like effector OmSSP1 was necessary to colonize *Vaccinium myrtillus* roots (Casarrubia et al., 2018). In *Trichoderma*, the hydrophobin-like TasHyd1 protein, isolated from *Trichoderma asperellum*, plays an important role in root attachment and colonization (Viterbo and Chet, 2006), while the knockout or overexpression of the class II hydrophobin TvHydII1 in *T. virens* resulted in the reduction or enhancement of root colonization, respectively (Guzmán-Guzmán et al., 2017). Furthermore, transcriptome analyses during the interaction of *T. virens* with maize roots revealed upregulation of several hydrophobin-like genes, including the *hfb7* and *tvhydii1* genes (Lawry, 2016).

Other proteins, which have been associated with planting communication, are the ceroplatanins. Sm1 is a small-secreted protein that is involved in ISR but which is dispensable for plant colonization. Interestingly, Sm2, a paralog of Sm1 is involved in root colonization, although the mechanism is currently unknown (Crutcher et al., 2015).

Role of phytohormones in plant defense

Phytohormones play key roles in the plant immune signaling network that is activated upon the perception of microbes. This primarily involves primary defense hormones, such as jasmonates (JA), ethylene (ET), and salicylates (SA), but secondary defense hormones, such as auxins (IAA), abscisic acid (ABA), cytokinins (CKs), brassinosteroids (BRs), and gibberellins (GA), also have a significant role, either alone or in conjunction with the primary phytohormones (Kazan and Lyons, 2014; Shigenaga and Argueso, 2016; Berens et al., 2017). Inside plant tissues, plant symbionts can synthesize a wide range of phytohormones to stimulate plant growth, induce tolerance to abiotic stress, and provide resistance to biotic factors (Egamberdieva et al., 2017).

Microbes have evolved mechanisms to manipulate hormone signaling pathways to spread into host tissues, colonize, and cause disease. In both pathogenic and beneficial microorganisms, these mechanisms may include the production of signaling molecules such as auxins, influencing the synthesis of secondary hormones that contribute to the suppression of primary hormonal signaling, or generating compounds that function as molecular mimics of the phytohormones (Kunkel and Brooks, 2002; Gimenez-Ibanez et al., 2014; Xu et al., 2015; Nobori et al., 2018). Beneficial microbes interact with the plant in a similar way to pathogens. For instance, *Laccaria bicolor*, a mutualistic symbiotic fungus, produces an effector-like protein MiSSp7 that acts as a negative regulator of JA, minimizing the impact of JA during the interaction of *L. bicolor* with roots (Plett et al., 2014). Plant growth promotion by *Trichoderma* has been attributed to either the production or induction of different kinds of phytohormones (Sofo et al., 2011). However, this effect can be induced by multiple factors or molecules, including volatile organic compounds or solubilization of micronutrients by *Trichoderma* and requires further study (Stewart and Hill, 2014; Garnica-Vergara et al., 2016; Nieto-Jacobo et al., 2017). Recently, the presence of potential encoding gene proteins associated with the synthesis of diverse phytohormones in *Trichoderma* (e.g., auxins, cytokinins, abscisic acid, gibberellins, etc.) was reported (Guzman-Guzman et al., 2019). However, the role of phytohormones produced by *Trichoderma*, specifically auxins and their role in plant growth promotion, has historically been misinterpreted and constantly cited as responsible for growth promotion (Contreras-Cornejo et al., 2009), even with multiple evidences suggesting no correlation between auxin production (in many cases, it was actually total indoles that were determined) and plant growth promotion (Hoyos-Carvajal et al., 2009; Nieto-Jacobo et al., 2017). Thus, a detailed study into these molecules is necessary to accurately evaluate their role in *Trichoderma* biology and their impact on plant defense.

Plant hormones play other important roles in the plant defense architecture during communication with fungi. For instance, plants require phytohormones to control microbe colonization. In the *A*rabidopsis *thaliana*–*Trichoderma* model, it was discovered that the host plant, without the support of SA, is unable to prevent the colonization of *T. harzianum* in the vascular system, leading to imminent plant death (Alonso-Ramirez et al., 2014). Moreover, GA

signaling regulates the expression of arbuscular mycorrhizal-induced genes that have a direct impact on hyphal entry and branching into the host root (Takeda et al., 2015). Further research is required, however, to elucidate the mechanisms by which beneficial microorganisms interact with the plant via the signaling pathways that involve the plant−microbe phytohormone network.

Role of secondary metabolites in plant−fungi interaction

Plants and microbes coexist in the same niche, establishing a plethora of interactions. Both beneficial and detrimental interactions are ruled by a broad variety of primary and SMs, including sugars, fatty acids, amino acids, nucleic acids, phenolic compounds, terpenoids and steroids, hormones, and compounds containing nitrogen or sulfur (alkaloids, glucosinolates, and nonribosomal peptides and proteins) (Pusztahelyi et al., 2015; Musilova et al., 2016). Many of these molecules fit into the category of SMs. In most cases, they are responsible for the transcriptome modifications in plants and microorganisms that are necessary to cope with environmental biotic and abiotic stress.

SMs are structurally heterogeneous, low molecular weight compounds, such as nonribosomal peptides, polyketides, terpenoids, and pyrenes (Maffei, 2010; Niu and Tan, 2013; Vivaldo et al., 2017). SMs are produced from primary metabolism molecules, amino acids, mevalonate, and acetyl coenzyme A derivatives, and in some cases secondary modifications are required (Zeilinger et al., 2016). Nonribosomal peptide synthase (NRPS) and polyketide synthase enzymes are the primary synthesizers of SMs. Other molecules, such as oxidases, transporters, and regulatory proteins (genes encoded mostly in the same cluster), are often involved in the regulation, modification, and synthesis of these metabolites, with the genes for their biosynthesis usually clustered, too (Brakhage and Schroeckh, 2011; Brakhage, 2013; Medema et al., 2015).

In nature, every organism produces chemically diverse SMs, and many of these could be shared with some taxonomically related or unrelated living organism (Verpoorte, 2000). Even though SMs are not involved in growth and development, they do provide survival advantages to the organisms producing them (Zeilinger et al., 2016). Here, the rhizosphere serves as a complex arena where plants and different microorganisms secrete SMs as major messengers to establish beneficial or detrimental relationships (Mhlongo et al., 2018). Species of the genus *Trichoderma* are known to produce a vast array of SMs, mainly when the fungi interact with other microorganisms in the rhizosphere (Mukherjee et al., 2012; Schmoll et al., 2016). Moreover, *Trichoderma* SMs participate both as defense molecules and as signal molecules to communicate with other organisms in the environment (Zeilinger et al., 2016).

Trichoderma metabolites have essential roles in a variety of cellular processes, such as intracellular communication, transcription, and development (Mukherjee et al., 2012), with the metabolome profile of *Trichoderma* spp. being incredibly complex as a result. The capability of this genus to produce a broad diversity of natural products has been undervalued, because many of the fungal SM biosynthesis gene clusters are not expressed under standard cultivation conditions (Mukherjee et al., 2012). For example, *tex1*, a NRPS gene, is encoded over 62,810 base pairs (Viterbo et al., 2007). Tex1 is involved in the production of 18mer peptaibols in *T. virens*, which are important metabolites involved in the induction of plant defense and with antimicrobial activity.

Trichoderma metabolites are involved in a number of important biological roles. For instance, several metabolites, such as phytotoxins, mycotoxins, pigments, and antibiotics, exhibit biological functions and play a significant role in the regulation of biological interactions, while others have been implicated in sporulation and hyphal elongation (Vinale et al., 2014). As the syntheses of SMs depend on biotic and abiotic factors, the production of a variety of these metabolites (e.g., peptaibols, polyketides, nonribosomal peptides, auxins, phenolic compounds, terpenoids, steroids, siderophores, pyrones, etc.) depends on the stimuli presented by the environment. Due to the nature of these compounds, some of these biological interactions can be potentially harmful, while others can reshape the metabolism and growth rate of plants (Lee et al., 2015, 2016; Malmierca et al., 2015; Garnica-Vergara et al., 2016; Coppola et al., 2017; Nieto-Jacobo et al., 2017). Such is the case with the 6-pentyl-α-pyrone (6-PP) molecule, which can regulate plant growth in a concentration-dependent manner (Garnica-Vergara et al., 2016), in addition to its fungistatic and antibiotic activity (Vinale et al., 2008a,b, 2014; Kumar et al., 2017). 6-PP has been reported to control seedling blight in maize plants (El-Hasan and Buchenauer, 2009), with preliminary experiments suggesting that the activation of defense mechanisms in treated plants occurs by increasing PO and polyphenoloxinadase (PPO) activity: POs are involved in the generation of ROS and lignification processes of cell walls (a plant defense mechanism), and PPO activity has been linked to the plant response to infection through increased production of phenolic compounds, such as phytoalexins, flavonoids, and coumarins, among others (El-Hasan and Buchenauer, 2009).

SMs can have other important roles as well. For instance, SMs which are part of the chemical defense of plants, such as alkaloids, coumarins, isoflavonoids, polyacetylenes, quinones, tannins, and terpenes, have antimicrobial activity (Singh and Sharma, 2015). They can also act as mediators of plant–microbe interactions and shape the composition of microbial communities and their metabolic pathways (Musilova et al., 2016). Most plant microbes have evolved to manipulate their host metabolism and induce favorable nutritional conditions. For instance, the fungal plant pathogen *Alternaria* secretes host-specific toxins that act as effectors to induce toxicity and pathogenicity (Friesen et al., 2008). In contrast, mutualistic-endophytic fungi secrete mycotoxins to protect the host plant against herbivores (Faeth, 2002). Likewise, root endosymbionts can also modify the plant metabolome, including SM levels in leaves, like catalpol levels, for example, which play a role in direct plant defense (Schweiger et al., 2014).

Effector proteins and plant immune repression

Plant pathogens, and most likely fungal endophytes, manipulate host cellular processes through the secretion of molecules named effectors. These are microbe-produced molecules that have specific effects on the genotype of their host, thereby enabling the microbes to establish a close relationship with the plant.

Effector molecules can be difficult to identify, as they have very little homology to most known proteins. So far, the only common sequence element seen in fungal effectors is the high number of cysteine residues, which has possibly evolved as a defense against plant proteases and the presence of an N-terminal signal peptide (around 20 amino acid residues) in secreted proteins dependent of the ER system (Stergiopoulos and de Wit, 2009). Recent

evidence suggests that conservation of structure may allow a better definition of effector groups (de Guillen et al., 2015). More is known of bacterial effectors, particularly those involved in the type III secretion system, which can now be identified via predictive tools (Arnold et al., 2009; McDermott et al., 2011; Wang et al., 2018a). Tandem repeats have been major predictors of effector proteins in diverse fungi, including *Trichoderma*, *Melampsora* spp., and *U. maydis* (Mesarich et al., 2015; Schmoll et al., 2016; Mendoza-Mendoza et al., 2018).

The majority of known effectors are secreted and are classified based on their localization in the plant cell. Apoplastic effectors are secreted in the apoplast and act as counter-defense molecules by inhibiting host enzymes, e.g., plant hydrolases and proteases (Morgan and Kamoun, 2007; Clark et al., 2018), whereas cytoplasmic effectors are translocated into the cell cytoplasm where they interact with intracellular targets to suppress the plant immune response (Kale, 2012; Shi et al., 2018). Many cytoplasmic effectors delivered by oomycetes carry an N-terminal signal peptide for secretion, followed by the RxLR motif that is in charge of the translocation process with the C-terminal domain having the biochemical activity associated with the effector (Schornack et al., 2009; Wang et al., 2017).

Diverse potential effector-like proteins from *Trichoderma* have been identified based on bioinformatic studies of conventionally secreted proteins (proteins containing an N-terminal signal peptide) and size (those proteins smaller than 300 amino acid residues) (Schmoll et al., 2016; Guzmán-Guzmán et al., 2017; Mendoza-Mendoza et al., 2018). A few of these effectors have RxRL motifs (Guzmán-Guzmán et al., 2017), but their roles still need to be determined. Some of these effectors are upregulated in *T. virens* during interactions with maize roots (Lawry, 2016). However, recently effector proteins of tandem repeats have been identified that are bigger than 300 amino acids, suggesting that the size is not a good characteristic to use to define effector proteins (Ma et al., 2018).

Proteins with the fungal-specific CFEM domain, containing eight cysteine residues, have proposed roles in fungal pathogenesis. These proteins include multifamily of proteins with both different lengths and, most likely, different and so far uncategorized motifs, beyond the CFEM domain and signal peptide (Mendoza-Mendoza et al., 2018). The role of these proteins has been linked to iron metabolism, cell wall stability, stress, and pathogenicity in some fungi (Okamoto-Shibayama et al., 2014; Vaknin et al., 2014; Zhu et al., 2017), and they comprise a diverse group in *Trichoderma* spp.

Hydrophobin proteins, like CFEM proteins, are secreted by fungi and are considered important elements in communication. They operate not only in the attachment of the hyphae to the plant surface (e.g., MPG1 from *M*agnaporthe *grisea*) (Beckerman and Ebbole, 1996; Talbot et al., 1996) but also in early and late signaling mechanisms during plant recognition of *Trichoderma* root colonization, activating proteins involved in early stages in the interaction (Guzmán-Guzmán et al., 2017; Casarrubia et al., 2018; Moscatiello et al., 2018). For example, in *Lotus japonicas,* the hydrophobin HYTLO1 from *Trichoderma longibrachiatum* induces Ca^{2+} signaling pathway activation through the modulation of nicotinic acid adenine dinucleotide phosphate (NAADP), a potent Ca^{2+} mobilizing messenger (Moscatiello et al., 2018). Furthermore, the hydrophobin ThHyd1 from *T. harzianum* interacts directly with the ubiquitin 1-like (UBL) from maize roots, and their overexpression in *A. thaliana* promoted plant resistance against *Botrytis cinerea*. This resistance most likely conferred via a mechanism associated with brassinosteroid signaling via BAK1 (Yu et al., 2019), although Jasmonate/ethylene (JA/ET) signaling has been also involved to some extent in this response. These results

suggest that the Hyd1-UBL axis might play a key role in inducing systemic resistance in *Trichoderma*–plant interactions (Yu et al., 2019). Like tvhfb7 (Przylucka et al., 2017), Tvhydii1 is a hydrophobin upregulated during the interaction between *T. virens* and *A. thaliana*. Genetic deletion of Tvhydii1 seems to suggest its role in root colonization, although further verification is required (Guzmán-Guzmán et al., 2017). In other fungi, like the ericoid mycorrhizal fungus *O. maius*, the hydrophobin OmSSP1 was reported as a potential effector during symbiosis with *V. myrtillus* roots (Casarrubia et al., 2018), although more experimental work needs to be done to confirm the exact role of this protein in the symbiosis.

Chitin detection is also important for fungal recognition during the invasion. Chitin is detected by plant surface receptors, such as the chitin receptors CEBiP and CERK1 on the plasma membrane, which have the chitin-binding lysin motif (LysM) in their ectodomains (Kombrink et al., 2017; Desaki et al., 2018). Recognition of chitin via these receptors activates plant defenses; however, fungi can overcome chitin recognition either by masking their cell wall surface, changing its chemical composition (e.g., from chitin to chitosan), or by secreting proteins that highjack the chitin released during the fungal growth inside the plant, thus evading detection (Kombrink et al., 2017). LysM effectors are released during plant colonization, with evidence to date indicating that these motifs bind chitin and are responsible for evasion of cell wall fungal perception during the root colonization. Interestingly, the expression and role of these proteins seem to be dependent on the host and in some cases are dispensable for host colonization (Kombrink et al., 2017). The role of these proteins in plant defense in *Trichoderma* has yet to be determined.

Effector research in *Trichoderma* is still in its infancy. Different orthologs encoding effector genes from plant fungal pathogens have recently been identified in *Trichoderma* spp. (for more information, see Supplementary Tables from Mendoza-Mendoza et al., 2018), but their role in the interaction with plants requires further study. For instance, the interaction between *T. virens* and maize roots induces a reduction in proteins associated with the metabolism of ROS and a subsequent reduction in PO activity in the apoplastic fluids (Nogueira-Lopez et al., 2018). The current mechanism proposed for this observation suggests that *T. virens* induces a reduction in the levels of scavenging ROS proteins, such as plant POs. The mechanism of this reduction is currently unknown but it most likely involves transcriptional or posttranscriptional regulation by *Trichoderma* on the plant host (Nogueira-Lopez et al., 2018).

ETI detects effector molecules by identifying modifications to plant signaling pathways or receptors, which in turn causes upregulation of many immune-related genes, and activates the HR (Cui et al., 2015). When resistance (R) proteins, which are usually nucleotide-binding site–leucine-rich repeat (NBS-LRR) proteins, bind to an effector molecule, they activate downstream responses, such as MAPK, volatile organic compound production, and the modification of plant hormone levels (Cui et al., 2015; Han, 2019). Sufficient levels of plant hormones, particularly SA, then lead to enhanced and systemic plant resistance to a particular pathogen (Carvalhais et al., 2017). This sequence of events, and the evolutionary significance of these interactions, has for a long time been modeled using the zig-zag model: a highly influential concept that describes the timing and evolution of this process (Fig. 5.2) (Jones and Dangl, 2006).

The zig-zag model implies four discrete stages of interaction. Firstly, PTI (or MTI) leads to a basal level of response. This is followed by the release of fungal effectors, which reduces the response to a level that is ineffective against the pathogen. MTI is then countered by ETI, which restores and amplifies the initial response. This ETI may then be countered by further

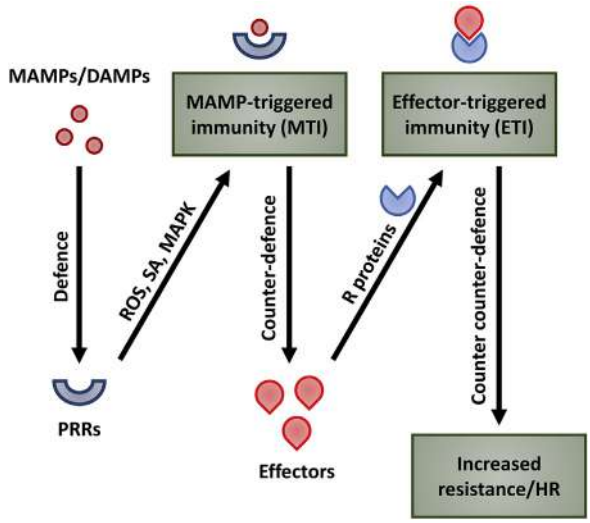

FIGURE 5.2 **Schematic Representation of Zig-Zag Model in Plant Immunity During Plant–Microbe Interactions.** In the first stage, microbe-/damage-associated molecular patterns (MAMPs/DAMPs) are detected via cognate pattern recognition receptors (PRRs) to initiate MAMP-triggered immunity. In the second stage, microbes deliver effector-like proteins to overcome MTI and establish a successful infection, resulting in effector-triggered susceptibility (ETS). In the third stage, host recognition of one or more effector-like proteins by disease resistance (R) proteins, activates effector-triggered immunity (ETI), which is a robust and accelerated immune response. In the last stage, continuous cycles of PTI, ETS, and ETI drive host–microbe coevolution in effector functions and effector recognition, which might result in disease resistance and, usually, a hypertensive cell death response (HR) at the infection site. Abbreviations: *MAPK*, mitogen-activated protein kinase; *ROS*, reactive oxygen species; *SA*, salicylic acid. *Image adapted from Incarbone, M, Dunoyer, P, 2013. RNA silencing and its suppression: novel insights from in planta analyses. Trends Plant Sci. 18, 382–392.*

effectors, which may then be countered in turn by ETI. This model has recently faced criticism, as it is proposed that the distinction between time scales may not be realistic in terms of direct molecular interactions (Pritchard and Birch, 2014). Correct identification of the timeframe of this process is critically important, as the timing of the interaction determines the rates of pathogen clearance or host death. It also suggests that the time between the phases of MTI and ETI is very small. The evolutionary scale is also important, but in a different context. This is because, for molecular work, the organism's evolutionary capability is unlikely to change across the duration of a particular experiment.

Role of iron during plant–microbe interactions

Iron (Fe) is an essential microelement present in the soil which is vital for animals, plants, and microorganisms. Iron participates as a cofactor in different metabolic processes, including respiration, photosynthesis, DNA repair, and the reduction of ribonucleotides and molecular nitrogen (Guerinot and Yi, 1994; Dunn et al., 2007). Moreover, iron homeostasis in living cells is crucial, as it is highly reactive and toxic, via ROS production, which initiates and mediates cell death (Dixon and Stockwell, 2014). Thus, organisms have evolved several strategies to maintain iron homeostasis, such as iron transport and storage, and the involvement of regulatory proteins (Wang and Pantopoulos, 2011).

Iron bioavailability in most soils on earth is limited (Guerinot and Yi, 1994). As a result, plants and microorganisms have evolved different strategies to favor acquisition of iron. Two main strategies have been widely studied in plants: strategy I, used by nongraminaceous monocots, based on the acidification of the rhizosphere to increase Fe^{3+} solubility through proton secretion, transplasma membrane electron transfer, and transport; and strategy II, which relies on phytosiderophore secretion and is only present in graminaceous monocots (e.g., maize) (Staiger, 2002). In contrast, microorganisms utilize three mechanisms to solubilize iron and incorporate it into the cell: (a) reduction of ferric ion (Fe^{3+}) to ferrous (Fe^{2+}) form, (b) acidification of the environment, and (c) excretion of soluble iron-chelating molecules (Philpott, 2006). Plants and root-associated microorganisms uptake iron mainly through the rhizosphere (Morrissey and Guerinot, 2009), where its incorporation via rhizosphere-associated microbes is essential in making iron acquisition more efficient to plants (Jin et al., 2014).

Iron is a key element during plant–microbe interactions (Lemanceau et al., 2009). For instance, in the pathosystem *U. maydis* maize, iron uptake via ferroxidase/permease system is fundamental for biotrophic development (Eichhorn et al., 2006), while in the phytopathogenic fungus *Cochliobolus heterostrophus*, siderophores production is necessary for full virulence (Lee et al., 2005). *Trichoderma* stimulates iron uptake responses that readjust iron homeostasis in roots, enhancing lateral root development and root hair proliferation (Martínez-Medina et al., 2017). Furthermore, in *T. asperellum*, iron and chlorophyll content in white lupin was influenced by the presence the fungus depending on the availability of iron in the media (de Santiago et al., 2009).

Siderophores are low molecular weight iron-chelating SMs, produced by many microorganisms and plants growing under limited iron conditions that can remain intracellularly or be secreted into the surroundings (Ahmed and Holmström, 2014). In the rhizosphere, iron competition is highly demanding, favoring those specialized microorganisms that have developed mechanisms, such as siderophores, to chelate and acquire iron by the production of high-affinity scavenging compounds (Jin et al., 2014). Siderophores synthetized by microbes have been shown to have beneficial effects to plants by enabling direct competition with pathogenic microorganisms, supressing their growth, and through iron solubilization (Leong, 1986). The majority of microbial siderophores belong to the hydroxamate-, carboxylate-, or catecholate-type (Saha et al., 2016).

Most fungi expertly uptake iron via the secretion of siderophores into the soil to chelate or bind available iron (Philpott, 2006). Fungi, including the genus *Trichoderma*, generally produce hydroxamate-type siderophores. Indeed, in *Trichoderma*, 18 different siderophores have been discovered from 10 different wild-type *Trichoderma* spp. (Lehner et al., 2013). The biocontrol *Trichoderma* sp., *T. virens* excretes 13 siderophores, including dimerum acid, coprogen, fusigen, ferricrocin, fusarinineA, fusarinine B, and des-diserylglycylferrirhodin (Lehner et al., 2013). The intracellular siderophore ferricrocin, which is expressed during iron-depleted conditions, is involved in oxidative stress regulation and SM biosynthesis (gliotoxin). Its deletion mutant in *T. virens* had a negative effect on the host plant by inhibiting root growth and demonstrating impaired ability to activate ISR (Mukherjee et al., 2018). The siderophore harzianic acid (HA), produced by *T. harzianum*, has positive effect on seed germination, and shoot and root growth in tomato, and iron content in the plants was increased when HA was supplemented under iron-deficient conditions (Vinale et al., 2013).

Additionally, HA showed antifungal activity against three different plant pathogens (Vinale et al., 2009). Siderophores can act as elicitors of ISR in plants (Lemanceau et al., 2009). For example, the siderophore pseudobactin, produced by the plant growth-promoting bacterium *Pseudomonas putida*, elicits ISR in *A. thaliana* (Meziane et al., 2005).

Iron can directly or indirectly contribute to plant defense mechanisms, depending on the modulation of iron supply during pathogen infection. Iron participates in the amplification of ROS production or in the metabolic activity of the plant through, for example, the production of antimicrobial compounds that require Fe-dependent enzymes for their synthesis (Aznar et al., 2015). In wheat, iron mediates the oxidative burst, the induction of PR proteins, and the formation of localized cell wall appositions during the infection of *Blumeria graminis* f. sp. *tritici*, where PR genes are transcriptionally regulated under intracellular iron depletion (Liu et al., 2007). Furthermore, iron redistribution and storage are defense mechanisms utilized by the host plant upon microbe colonization, with the perturbation of plant iron homeostasis induced by pathogens being a common strategy to disturb host immunity (Fig. 5.3) (Verbon et al., 2017).

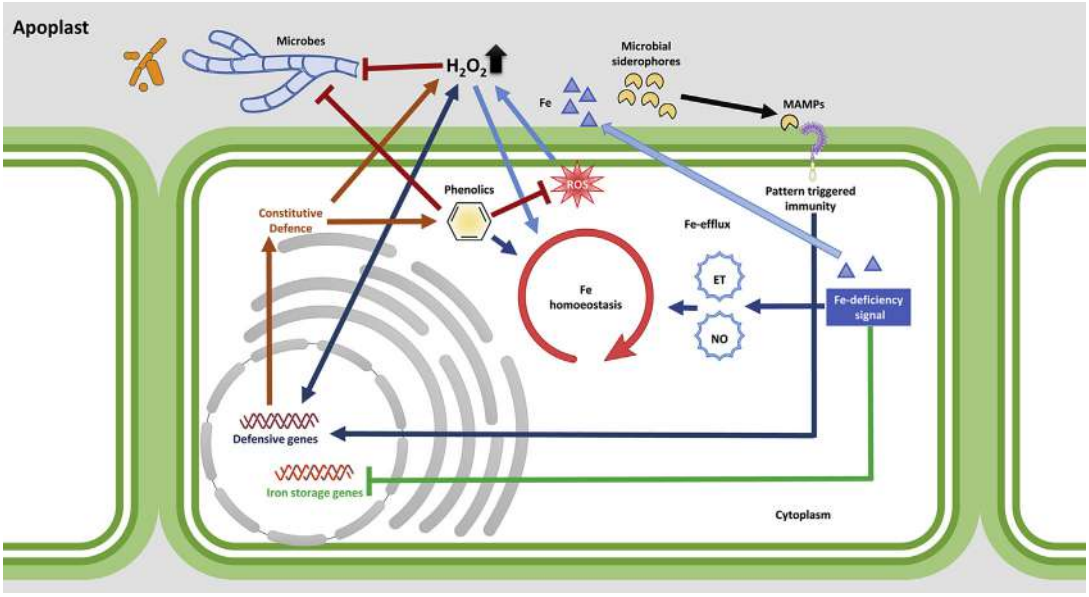

FIGURE 5.3 **Iron Homeostasis in Plant–Microbe Interactions.** Mobilization and redistribution of iron in the plant cytoplasm and apoplast when encountering microbes. Secretion of microbial siderophores in the rhizosphere triggers an iron deficiency signal and elicit pattern-triggered immunity via MAMPs, which activate plant defense responses such as ROS formation and accumulation of phenolic compounds. Iron efflux from the cytoplasm to the apoplast in colonized cells leads to an iron deficiency inside those cells, which triggers an oxidative burst in the apoplast caused by this accumulation of iron. In addition, phenolic compounds having antioxidant and antimicrobial properties contribute to the protection of the plant cell from ROS and pathogens. ET and nitric oxide (NO) are involved in defense signaling that influences iron homoeostasis. Induction of defense-related genes and suppression of iron storage–related genes are caused by extracellular H_2O_2 accumulation and iron deficiency. *Figure modified from Liu, G, Greenshields, DL, Sammynaiken, R, Hirji, RN, Selvaraj, G, Wei, Y, 2007. Targeted alterations in iron homeostasis underlie plant defense responses. J. Cell Sci. 120, 596–605; Aznar, A, Chen, NWG, Thomine, S, Dellagi, A 2015. Immunity to plant pathogens and iron homeostasis. Plant Sci. 240, 90–97.*

Conclusions and summary

Understanding the molecular dialogue between plants and mutualists is essential to the safe and effective use of BCAs such as *Trichoderma* in agriculture. While these fungi are capable of increasing crop yields and protecting crops from biotic and abiotic stress factors, the lack of a clear explanation for the difference between pathogenic and endophytic lifestyles is troubling. Similar molecules seem to be involved in both pathogenic and mutualistic interactions.

An in-depth study of *Trichoderma* in interaction with an agriculturally relevant plant will increase our understanding of the process of colonization and the differences between endophytism and pathogenicity (Harman et al., 2004). Pathogenic organisms have been well studied; however, the focus with beneficial microbes has mainly been on their antibiotic properties. *Trichoderma* spp. are good candidates as they exist as mutualists with agriculturally relevant plants and are already in use in agriculture, making findings readily applicable.

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