CHAPTER

1

Trichoderma in the rhizosphere: an approach toward a long and successful symbiosis with plants

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Introduction

In their natural settings, plants are constantly exposed to hostile conditions including biotic and abiotic stresses. Plants interact with a plethora of microorganisms leading to beneficial or deleterious interaction, unleashing plant defense mechanisms, including constitutive and induced resistance. Constitutive resistance is also referred as the nonhost-resistant mechanisms to sense and to resist the attack from a great proportion of pathogens. This first defense mechanism includes a structural layer and chemical constitutive. The structural layer is part of plant physical barriers, including the cuticle, trichomes, and waxes. The constituent chemicals are toxic compounds secreted by plant cells. Once microorganisms overcome the plant physical barriers, the plant defense systems are triggered, with the subsequent attempt of microorganism to suppress plant immunity to succeed in diseases or to set up a beneficial relationship (Yang and Huang, 2014). In addition to constitutive defense, plants have evolved additional immune layers, including the Microbial or Pathogen-Associated Molecular Patterns (MAMPs or PAMPs, respectively) trigger immunity (PTI). After recognition of MAMPs or PAMPs through cell surface–localized pattern recognition receptors, plants synthesize secondary metabolites (SMs) with antimicrobial activity such as phytoalexins, accumulate hydrolytic enzymes, and reinforce the plant cell wall by deposition of callose (Hématy et al., 2009). It is well-known that PAMPs and MAMPs induce the systemic acquired resistance (SAR) through the phytohormone salicylic acid (SA), which is highly accumulated at the infection site, and subsequently the signal spreads to distant parts of the original site of infection. The SAR signaling induction parallels well with the expression of a set of genes coding for proteins related to pathogenicity (PR) (Pieterse et al., 1996). SAR signaling is effective against biotrophic and hemibiotrophic microorganisms such as the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*DC3000) and the fungal pathogen *Colletotrichum* spp. (Hammond-Kosask and Jones, 1997). Moreover, beneficial microorganisms boost the induced systemic resistance, which is dependent on the synthesis of the phytohormones jasmonic acid and ethylene (JA/ET). In this case, *LOX-2* and *PDF1.2* genes, which code for the lipoxygenase-2 and a plant defensin, respectively, are induced in *Arabidopsis* (Jakob et al., 2007). The JA/ET signaling is effective against the necrotrophic pathogens such as the fungus *Botrytis cinerea*. Additionally, SA and JA/ET pathways can affect each other, either negatively or synergistically. The cross-talk between SA and JA/ET pathways provides potential for the activation of multiples resistance mechanisms, driving plants to prioritize a particular defense pathway against the attacker (Pieterse and Van Loon, 2004). On the other hand, pathogens have evolved effector molecules to suppress the PTI and succeed in disease into the host. In response, plants have developed resistance proteins that recognize effectors to promote

effector-triggered immunity (ETI) (Chisholm et al., 2006). During ETI, plants trigger the hypersensitive response (HR), a form of programmed cell death at the site of infection to delimit the pathogen and prevent its spreading to other parts of the plant (Nomura et al., 2005). Furthermore, after the plant is attacked by a pathogen or a beneficial microbe colonizes the root, plants show strong defense abilities against further pathogen infections. This response is the so-called "priming" (a hypersensitized state), which is a faster and more efficient response, compared with plants that were not attacked by pathogens or colonized by beneficial microorganisms (Conrath et al., 2006).

Fungi of the *Trichoderma* genus are filamentous ascomycetes, inhabitants of many ecosystems including forests, grassland, deserts and agricultural soils, among others (Kubicek et al., 2008). *Trichoderma* is commonly found in soil, decaying wood or colonizing the plant root surface. Moreover, some *Trichoderma* species are considered as excellent model systems for photomorphogenesis or regeneration (Casas-Flores et al., 2004; Hernandez-Onate et al., 2012). Furthermore, in the rhizosphere *Trichoderma* suppress or inhibit the growth of soil phytopathogens through the activation of multiple mechanisms, including mycoparasitism, synthesis of antibiotics, and competition for nutrients against plant deleterious microorganisms (Brotman et al., 2008; Shoresh et al., 2010). These beneficial effects that *Trichoderma* spp. provide to plants, and the fact that such fungi are a friendly option for the environment, make them one of the best alternatives for the biological control of plant diseases. The stages of the association and recognition of the fungus with its plant symbiont partner include the detection, recognition, contact, penetration of the plant roots, and evasion of plant-secreted toxins (Hermosa et al., 2012). During root colonization, *Trichoderma* uses different strategies to warrant its success in the establishment of a symbiotic relationship. Root colonization by *Trichoderma* led to plant growth of herbaceous, ornamental, forestry plants and model plants including *Arabidopsis* (Gravel et al., 2007; Hohmann et al., 2012; Salas-Marina et al., 2011). The plant growth induced by *Trichoderma* could be explained by the synthesis of phytohormone-like molecules, nutrients solubilization in the soil, increased intake of nutrients, and phytohormone modulation by the fungus (Harman et al., 2004a; Sofo et al., 2011; Estrada-Rivera et al., 2019). Furthermore, plants treated with *Trichoderma* show increased tolerance to biotic and abiotic stresses (Shoresh et al., 2010). In this regard, *Trichoderma* activates the plant systemic resistance against phytopathogens by the induction of the SA and JA/ET signaling pathways, probably through MAMPs or effectors. These molecules can be proteins, nonribosomal peptides (peptaibols), SMs, or volatile organic compounds (VOCs) (Shoresh et al., 2010; Hermosa et al., 2012; Estrada-Rivera et al., 2019). Moreover, *Trichoderma* has the ability to prime plants against subsequent attacks by pathogens (Salas-Marina et al., 2011, 2015). Several strategies have been used to unravel the molecular mechanisms involved in this complex interaction including genomics, proteomics, and, more recently, metabolomics. During the last few years, an emerging mechanism on the regulation of the interaction between *Arabidopsis* and *Trichoderma* has been approached, where chromatin remodelers and small RNA pathways have been considered as modulators of such beneficial interaction (Ramírez-Valdespino et al., 2019; Rebolledo-Prudencio et al., under review). In this chapter, we will focus on *Trichoderma*-plant interaction, highlighting processes such as root colonization, promotion of plant growth, induction of plant systemic diseases resistance,

the contribution of priming to biotic and abiotic stress resistance, putative effector-like molecules, as well as emerging mechanisms that govern such interaction.

Plant root colonization by *Trichoderma*

The rhizosphere is the area of soil directly affected by the plant's radicular system. There are three different associations established in this environment classified as positive, negative, or neutral. The neutral plant–microbe interactions are one of the most common associations found in nature, where microbes utilize plant-derived organic compounds as sources of energy and play a key role in nutrient cycling (Schenk et al., 2012). Negative plant–microbe interactions include competition or parasitism by bacteria, fungi, or invertebrate herbivores, whereas positive plant–microbe interactions include symbiotic associations with mycorrhizal fungi or plant growth–promoting bacteria and fungi (PGPB and PGPF, respectively) (Bais et al., 2006). Signaling in the rhizosphere between plant and microbes has been widely studied, and root exudates (REs) play a major role in determining the outcome of the interaction (Bais et al., 2006). REs can be high- or low-molecular-weight compounds released or secreted by the plant roots. Low-molecular-weight exudates comprise amino acids, organic acids, sugars, phenols, and other SMs, whereas high-molecular-weight exudates comprise mucilage (polysaccharides) and proteins (Marschner, 1995). Some *Trichoderma* strains establish plant-positive interactions in the soil. Interestingly, the role of REs in plant–*Trichoderma* interaction has been scarcely studied. Recently, it was found that peroxidases and oxylipins can act as chemical signals in the soil, able to attract or stimulate the growth of the beneficial fungus *Trichoderma harzianum* (Fig. 1.1) (Lombardi et al., 2018).

Trichoderma rhizosphere–competent strains must possess the ability to grow and exert their function as beneficial microorganisms to plants in the developing rhizosphere (Ahmad and Baker, 1987; Harman, 2000). For example, *T. harzianum* mutants are rhizosphere-competent in the presence of benomyl in the soil, improving its ability to promote seedling emergence and plant growth (Ahmad and Baker, 1987), whereas *T. harzianum* fusing protoplasts of auxotrophic mutants increase its ability to colonize the entire root of maize plants (Sivan and Harman, 1990).

To provide its beneficial effects to plants, *Trichoderma* strains must be capable of colonizing the plant roots. During the plant root colonization by *Trichoderma*, the fungus grows into the epidermis and outer cortex, mainly through intercellular spaces of cucumber roots, which at the same time is coupled to epidermal and cortical cell strengthening (Yedidia et al., 1999). *T. harzianum* strains expressing the green-fluorescent protein (GFP) lead to the discovery of the papilla-like hyphal tips formation in this fungus during colonization of tomato roots (Chacón et al., 2007). Moreover, *Trichoderma atroviride* forms appressorium-like structures during its coculture with *Arabidopsis* and the hyphae grow into the intercellular spaces of the root epidermis (Salas-Marina et al., 2011). Interestingly, *Trichoderma virens* not only grows into the intercellular spaces of maize roots but also inside the cells. These evidences suggest that the fungus utilizes intra- and intercellular growing processes to establish itself in the root system (Fig. 1.1) (Nogueira-Lopez et al., 2018). Plant-derived sucrose has been defined as a key element in the symbiotic association between *Trichoderma* and plants (Koch, 2004; Vargas et al., 2009). In this regard, an intracellular invertase (an enzyme that catalyzes *the hydrolysis of sucrose into fructose and glucose*) from *T. virens* (TvInv) is crucial for the transport and

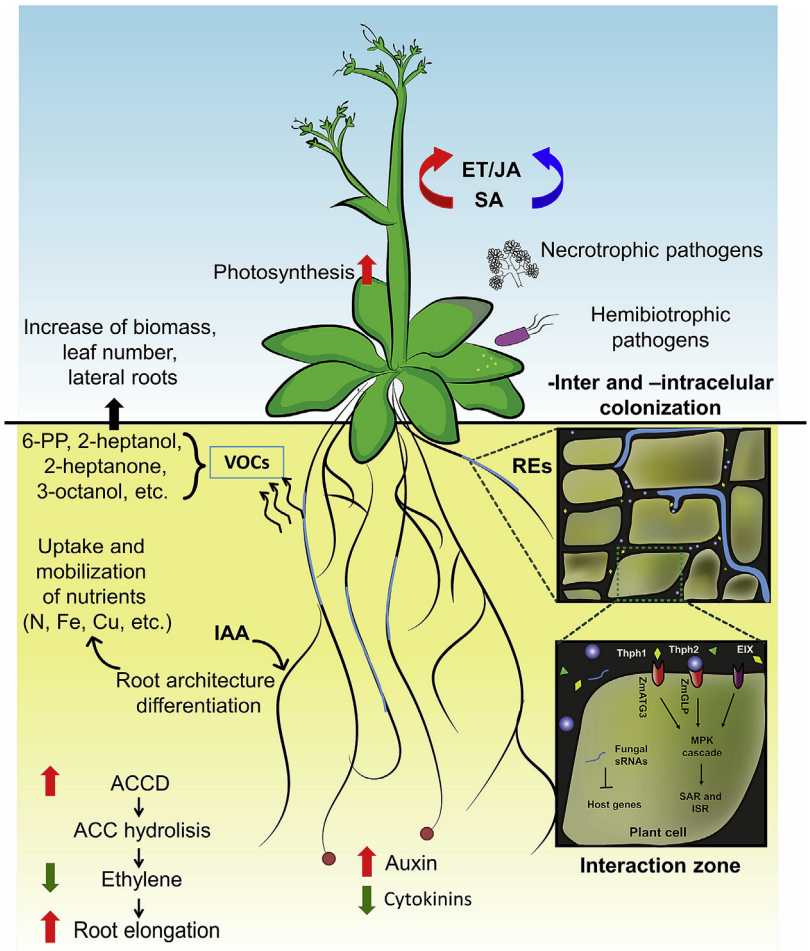

FIGURE 1.1 ***Trichoderma*—Plant Interaction.** Prior to the physical interaction of *Trichoderma* with the plant, the root exudates (REs), including peroxidases and oxylipins, act as chemical signals in the soil to stimulate the growth of the mutualistic fungus. When *Trichoderma* enters in contact with the plant, it is recognized as an alien, triggering the plant immunity locally and systemically. During plant root colonization, *Trichoderma* grows in between the intercellular spaces as well as inside the plant cells. Consequently, *Trichoderma* releases different elicitors such as Thph1 and Thph2 that are recognized by the root receptors *ZmATG3* and *ZmGLP*, triggering the plant systemic disease resistance. The EIX (ethylene-inducing xylanase) elicitor also induces the systemic disease resistance through the plant MAPK cascade, independently of its enzymatic activity. To counteract the plant defenses, probably, *Trichoderma* uses effector molecules including proteins and small RNAs to damp the host immunity—related genes to settle down inside the plant roots. Thereafter, *Trichoderma* upregulates the carbohydrate metabolism and the photosynthesis-related genes. The fungus also modulates the signaling and synthesis of phytohormones including Auxins, Ethylene, Jasmonic Acid, and Cytokinins. During root colonization, *Trichoderma* promotes the synthesis and accumulation of Auxins at the root tips (red circles), whereas the accumulation of cytokinins decreases. Together, these events correlate with the shortening of the primary root, and an increase in lateral roots number. Furthermore, *Trichoderma* induces the activity of ACCD (1-aminocyclopropane-1-carboxylate deaminase), which leads to ACC (1-aminocyclopropane-1-carboxylic acid, the ethylene precursor) hydrolysis, leading to a decrease in ethylene production, enhancing root elongation and plant growth. The synthesis of the auxin indole-3-acetic acid (IAA) by *Trichoderma* promotes the plant growth and the emergence of secondary roots improving the root surface area and consequently the uptake and mobilization of nutrients. All these facts occur either by direct contact of the fungus with the plant roots or by the emission of volatile organic compounds (VOCs, i.e., 6-PP and 2-heptanone). In summary, the modulation of the different phytohormone pathways by *Trichoderma* confers many beneficial effects to plants, including the priming against necrotrophic and hemibiotrophic pathogens.

utilization of sucrose released by plants and to establish a symbiotic association with maize seedling. Strains lacking *TvInv* are affected in their ability to grow in sucrose as carbon source. In addition, *TvInv* lacking strains show higher levels of root colonization, correlating with a stronger production of cell wall–degrading enzymes (Vargas et al., 2009). Moreover, a plant-like sucrose transporter was identified in *T. virens* (TvSut), suggesting an active sucrose transference from the plant to fungal cells during their mutualistic interaction (Vargas et al., 2011). TvSut from *T. virens* is a highly specific sucrose/H^+ symporter induced at early stages of root colonization (Vargas et al., 2011). *Trichoderma* root colonization is a complex process, which follows a series of relevant steps including root adherence and plant root penetration. In *Trichoderma asperellum*, TasHyd1, a hydrophobin of the class I mediates root surface adherence. Lack of *TasHyd1* gene impairs root attachment and colonization of cucumber roots (Viterbo and Chet, 2006). Furthermore, in *T. asperellum* an expansin-like protein, which bears a cellulose-binding domain able to recognize cellulose and modify plant cell wall architecture, is necessary for root colonization as well (Brotman et al., 2008). Plant cell wall–degrading enzymes are also required in *Trichoderma* for active root colonization. For instance, a *T. harzianum* mutant in *Thpg1* gene, which codes for an endopolygalacturonase, is also affected in plant-root colonization (Morán-Diez et al., 2009).

Once inside the roots, *Trichoderma* spp. must be able to suppress and/or tolerate the plant defense mechanisms. For instance, *T. harzianum* SQR-T037 strain is able to degrade phenolic compounds isolated from cucumber rhizosphere, including 4-hydroxybenzoic acid, vanillic acid, ferulic acid, benzoic acid, 3-phenylpropionic acid, and cinnamic acid (Chen et al., 2011).

The ATP-binding cassette transporter cell membrane pump TAABC2 from *T. atroviride* works as an extensive and powerful cell detoxification system in the fungus, supporting the role of ABC2 in root colonization, helping the fungus to withstand adverse environmental conditions (Ruocco et al., 2009). Recently, a mutant lacking the histone deacetylase HDA-2 of *T. atroviride* resulted in a diminished resistance against reactive oxygen species (Osorio-concepción et al., 2017). Moreover, Δ*hda-2* is impaired in its ability to colonize *Arabidopsis* roots, affecting the plant growth promotion effect and the plant defense response modulation provided by the fungus, highlighting the importance of chromatin modifications in root colonization by *Trichoderma* (Estrada-Rivera et al., 2019). The plant defense responses also play important roles in the root colonization process, which complicates the understanding of root colonization in the field of *Trichoderma*–plant interaction. Root inoculation of *Arabidopsis* seedlings with *Trichoderma asperelloides* T203 led to the upregulation of *WRKY18* and *WRKY40* transcription factors encoding genes, whose products stimulate JA-signaling via suppression of JAZ repressors. The *Arabidopsis wrky18/wrky40* double mutant resulted in reduced root colonization by T203, suggesting the importance of this pathway for the proper colonization of *Arabidopsis* roots (Brotman et al., 2013). Not only JA but also SA pathway is relevant for root colonization. In the absence of a functional SA pathway, plants are unable to prevent the invasion of the vascular system by the fungus, including the aerial parts, leading to the plant collapse (Alonso-Ramírez et al., 2014). In this regard, the endophytic strain *T. harzianum* T-78 induces the SA- and JA-regulated genes. Root-colonization of *sid2* (SA biosynthesis-impaired mutant) and *dde2-2* (JA biosynthesis-impaired mutant) plants by T-78 is faster in *sid2* lines, whereas JA-regulated pathway does not display an important role in root colonization by T-78 (Martínez-Medina et al., 2017a). *Trichoderma* spp. display mainly plant-positive interactions in the soil. The dissection of the immune response

triggered by SA and JA/ET pathways and the analysis of multiple *Trichoderma* mutants essential for plant-root colonization have been critical for the understanding of the colonization process. However, further efforts are required to elucidate the role of the REs, a novel research area that will help us to understand the plant−*Trichoderma* interaction at the rhizosphere level.

Trichoderma as plant-growth promoter

The capability of *Trichoderma* spp. to promote the growth of plants has been studied for a long time and is one of the most important traits of these fungi to be classified as beneficial to plants. Some of the first studies point out that after the application of *Trichoderma* spp., a significant increase in biomass of tomato, radish, and lettuce is observed (Lindsey and Baker, 1967; Baker et al., 1984; Ousley et al., 1994). Moreover, *T. harzianum* increases the growth and rooting and accelerates flowering after its application in several flower species as alyssum, marigold, pepper, periwinkle, and petunia, under commercial conditions (Baker, 1988). Growth promotion by *Trichoderma* is induced after it colonizes plant roots involving SMs produced by the fungus, including VOCs (Salas-Marina et al., 2011; Olmedo-Monfil and Casas-Flores, 2014; Estrada-Rivera et al., 2019). Today it is known that *Trichoderma* promotes plant growth by synthesizing phytohormone-like compounds and vitamins, enhancing the solubilization, uptake, and translocation of nutrients in soil, improving root development and increases in the rate of carbohydrate metabolism and photosynthesis (Olmedo-Monfil and Casas-Flores, 2014; Stewart and Hill, 2014). However, despite this topic has been extensively studied, the molecular mechanisms that govern this process are not completely understood. Here, we will discuss the most important findings of the plant growth promotion mediated by *Trichoderma* so far.

Plant growth promotion by *Trichoderma* strains

The induction of plant growth by *Trichoderma* strains has been extensively studied in many plants, including crops of agricultural importance. For instance, the colonization of tomato roots by *T. atroviride* and *T. virens*, and rice by seven *Trichoderma* spp. isolates (SL1-SL7), increases the fresh and dry weights, as well as leaf and tiller number, root length, and root fresh weight (Salas-Marina et al., 2015; Doni et al., 2014). This latter was correlated with an increase of photosynthetic rate and water use efficiency (Doni et al., 2014). Moreover, inoculation of *Arabidopsis thaliana* with *T. atroviride* and *T. virens* promotes seedlings growth, which correlates with bigger plants, an increase in biomass gaining and lateral root number (Contreras-Cornejo et al., 2009; Salas-Marina et al., 2011). Interestingly, studies on plant transcriptome reprogramming in response to different species of *Trichoderma* have shown that these fungi regulate genes involved in photosynthesis, nutrient transport, and hormone metabolism and signal transduction including ethylene and auxin. These genes together may induce the root architecture modification and by consequence induce the plant growth (Manganiello et al., 2018; Zhou et al., 2018; De Palma et al., 2019; Yuan et al., 2019).

It is noteworthy that not all *Trichoderma* strains are capable of inducing plant growth, even some authors have classified a number of strains as promoting, neutral, and negative (Lee et al., 2016). Also, a differential response according to plant genotype has been reported in maize and tomato (Harman et al., 2004b; Tucci et al., 2011).

Growth promotion by *Trichoderma* volatile organic compounds

VOCs are low molecular mass chemicals, usually hydrophobic that can easily evaporate at room temperature due to their high vapor pressure (Herrmann, 2011). *Trichoderma* species produce many SMs, including VOCs with potential agricultural importance (Mukherjee et al., 2012b). The main studies on VOCs have been focused on their functions as inductors of resistance to plant pathogens; however, during the last years, their function as growth promoters has taken importance. In this regard, *A. thaliana* plants growing together with *T. viride* under the same atmosphere, without direct physical contact, were taller, bigger, flowered earlier, had more lateral roots, and also increased their total biomass and chlorophyll content. Furthermore, the fungus produced 51 different VOCs, resulting the most abundant isobutyl alcohol, isopentyl alcohol, and 3-methylbutanal (Hung et al., 2013). Likewise, *T. virens*, *T. atroviride* (LU132 and IMI 206040), and *T. asperellum* LU1370 VOCs also increase the total biomass, shoot and root branching of *A. thaliana* seedlings (Contreras-Cornejo et al., 2014b; Nieto-Jacobo et al., 2017). For *T. virens* Gv29-8, it is known that sesquiterpenes are the most abundant VOCs produced and that the 4′-phosphopantetheinyl transferase (4′-PPT) is required for their synthesis (Contreras-Cornejo et al., 2014a).

Plant growth promotion by *Trichoderma* VOCs is dependent of both the plant and fungal culture age and is also influenced by environmental growth conditions (Lee et al., 2015; Nieto-Jacobo et al., 2017; Estrada-Rivera et al., 2019). *A. thaliana* seedlings of 7-day-old exposed to 5-day-old cultures of *T. atroviride* significantly increase shoot fresh weight and total chlorophyll content, whereas seeds exposed to 5-day-old cultures of the fungus, when germinated presented a reduction in plant size and less chlorophyll. Furthermore, the fungal cultures of 14-day-old produced more VOCs than 5-day-old cultures, including some alcohols, sesquiterpenes, and ketones; however, propyl acetate, pentyl acetate, and 2-heptanol were produced only by 5-day-old cultures (Lee et al., 2015). Moreover, Lee et al. (2016) reported nine *Trichoderma* strains whose VOCs promote plant growth. Of these, *Trichoderma pseudokoningii* (CBS 130756) VOCs are the most effective in promoting *Arabidopsis* plant growth, due to it induced the highest increase in fresh shoot weight and chlorophyll content. Similar results were observed in tomato plants exposed to *T. viride* VOCs. Several VOCs produced by this strain were identified as plant growth promoters, including ethyl 2-methylbutyrate, octadecane, 3-methylbutyl propanoate, (2E, 4E)-2,4-heptadienal, as well as a larger number of terpenes such as β-acoradiene, β-cubebene, β-cedrene, β-bisabolene, β-himachalene, and γ-himachalene. Furthermore, the VOC 1-decene (alkene), that it is known to be produced by *Trichoderma* spp., increases fresh shoot weight and chlorophyll content of *Arabidopsis* plants (Lee et al., 2019). On the other hand, the VOCs of *T. atroviride* (IMI 206040) 2-heptanol, 2-heptanone, and 3-octanol increase the dry weight of *Arabidopsis* seedlings (Fig. 1.1) (Estrada-Rivera et al., 2019). Interestingly, exposure of *Arabidopsis* to VOCs of a *T. atroviride Δhda-2* (histone deacetylase-2) mutant provokes enhanced plant growth

compared to its parental. Moreover, the lack of *hda-2* impaired VOCs production, resulting in an overproduction of 6-n-pentyl-6H-pyran-2-one (6-PP) (Estrada-Rivera et al., 2019). 6-PP is the most studied VOC produced by some *Trichoderma* strains and it induces plant biomass gaining, regulates the root architecture and formation of lateral roots, but its effect is dependent on the age of the plant and how it is applied (Vinale et al., 2008a,b; Garnica-Vergara et al., 2016; Estrada-Rivera et al., 2019). Moreover, it has been proposed that 6-PP acts as an effector molecule manipulating the physiology of the plant to promote the growth and emergence of secondary roots (Estrada-Rivera et al., 2019). In this sense, the fact that VOCs are low-molecular-weight chemicals, which evaporate, diffuse, and cross the membranes, makes them good candidates as signaling molecules to activate different plant responses as growth promotion, growth inhibition, and immune responses (Bitas et al., 2013; Bailly et al., 2014; Tyagi et al., 2018; Estrada-Rivera et al., 2019). It has been suggested that *Trichoderma* VOCs have a most relevant role in growth and plant development, whereas colonization of roots takes a minor role (Estrada-Rivera et al., 2019). In summary, *Trichoderma* VOCs seem to be key molecules that allow several *Trichoderma* species to be plant-beneficial fungi.

Mechanisms of plant growth promotion

Trichoderma is capable of modulates the plant hormone synthesis, enhances the solubilization and uptake of nutrients, promotes root development, and increases the photosynthetic and energetic efficiencies. These are the main traits through which these fungi promote plant growth (Yedidia et al., 2001; Harman et al., 2004a; Contreras-Cornejo et al., 2009; Salas-Marina et al., 2011). For instance, *Trichoderma* induces the upregulation of carbohydrate metabolism and photosynthesis in a number of plants (Fig. 1.1), including tomato treated with *T. harzianum* 6776, which improves the photosynthetic activity, correlating with enhanced growth promotion, fresh and dry weight gaining, and greater accumulation of pigments (Fiorini et al., 2016). In maize seedlings colonized by *T. virens*, the photosynthetic rate as well as transcripts of rubisco small subunit and the oxygen-evolving enhancer 3-1 are increased in leaves; being these process dependent of *T. virens* invertase, a glycoside hydrolase involved in sucrose degradation (Vargas et al., 2009).

On the other hand, the phytohormone IAA modulates the plant growth and root morphogenesis (Fig. 1.1) (Vessey, 2003). *T. atroviride* produces indole compounds related to IAA and their biosynthesis is stimulated by L-tryptophan *in vitro*. This fact correlates with an increase in fresh weight of shoot and roots of tomato and *Arabidopsis* plants, as well as an increase in the number of fruits in tomato. Contrastingly, this effect of growth was related with IAA degradation by *Trichoderma* in the rhizosphere and/or by their ACC deaminase activity (Gravel et al., 2007; Salas-Marina et al., 2011). In this regard, the inoculation of *Arabidopsis* seedlings with *T. virens* increased the expression of the auxin reporter *DR5::GUS*, whereas *Arabidopsis* mutants in genes involved in auxin transport or signaling (*aux1-7*, *doc1*, *eir1*, and *axr1-3*) showed diminished or null response to *T. virens* (Contreras-Cornejo et al., 2009). The induction of auxin-related genes as well as the accumulation of auxins in the root tips by *Trichoderma* VOCs also occurs (González-Pérez et al., 2018; Lee et al., 2019; Estrada-Rivera et al., 2019), indicating that either direct contact or VOCs can promote plant

growth via the modulation of auxin signaling pathway (Fig. 1.1). Moreover, the fact that 6-PP could be acting as an effector modulating the auxin pathway to promote growth and emergence of lateral roots has been proposed (Estrada-Rivera et al., 2019). The plant phytohormones cytokinins are involved in plant shoot growth but inhibit root growth (Werner et al., 2001). Inoculation of cherry and melon plants with *Trichoderma* growth-promoter strains (T-10, T-17 and T-22) causes a reduction of cytokinins in shoots, which correlates with an enhanced root growth (Fig. 1.1) (Sofo et al., 2011; Martínez-Medina et al., 2014). Furthermore, a higher concentration of ethylene correlates with plant root inhibition (Abeles et al., 2012). It is known that plant growth−promoting rhizobacteria produce 1-aminocyclopropane-1-carboxylate deaminase (ACCD), which hydrolyzes the ACC necessary for ethylene biosynthesis (Todorovic and Glick, 2008). In this regard, the silencing of *Tas-acdS* gene in *T. asperellum* reduces the capability of the fungus to induce root elongation of canola seedlings (Viterbo et al., 2010). Moreover, low levels of the ethylene precursor ACC is observed after inoculation of melon plants with *T. harzianum* T3, T7, and T22, which is consistent with an enhancement of plant growth (Fig. 1.1) (Martínez-Medina et al., 2014).

For a long time, the capability of *Trichoderma* spp. to enhance solubilization and uptake of nutrients has been studied. For instance, *T. harzianum* strain SQR-T037 increases the dry weight of tomato seedlings and copper (Cu) uptake under Cu-deficient conditions (Li et al., 2015). Likewise, inoculation with *T. asperellum* T42 increases total nitrogen (N) content in tobacco roots (Singh et al., 2018). It is well known that root architecture influences the nutrient uptake, through increased root distribution, especially root hairs, to cover more surface area (Fig. 1.1) (White et al., 2013; Aceves-García et al., 2016). The treatment of tobacco plants with *T. asperellum* T42 impacts positively in root differentiation, primary root length, root dry weight, and number of lateral roots (Aceves-García et al., 2016). Interestingly, under iron (Fe) deficiency, the MYB transcription factors MYB10 and MYB72 are rapidly upregulated in *Arabidopsis* roots, stimulating Fe mobilization and uptake from soil. Also, *myb10myb72* double mutants contain lower iron and chlorophyll levels, and shoot mass under such conditions, showing the crucial role of these transcription factors in plant growth and survival of *Arabidopsis* under iron-limiting conditions (Palmer et al., 2013; Zamioudis et al., 2014). In agreement with these results, the colonization of *Arabidopsis* roots by *T. asperellum* T-34 activates the expression of *MYB72* (Segarra et al., 2009), whereas *T. asperellum* and *T. harzianum* VOCs trigger the expression of such gene along with other Fe deficiency−related marker genes (*FRO2* and *IRT1*). This fact parallels well with morphological changes in root architecture, including the stimulation of root branching, development of root hairs, and root swelling in *Arabidopsis* and tomato seedlings, suggesting a role for MYB72 in plant−*Trichoderma* interaction to promote changes in root architecture (Martínez-Medina et al., 2017b).

All these mechanisms appear to be regulated by mitogen-activated protein kinase (MAPK) signaling pathways. Recent reports point out that once plants recognize *Trichoderma*, the chemical communication is activated via MAPK *in planta*. After this cascade is activated, processes of phosphorylation/dephosphorylation are carried out to activate or deactivate several transcription factors (Tyagi et al., 2018). This, in turn, induces differential responses, including those involved in growth promotion. Contreras-Cornejo et al. (2015) reported that inoculation of *Arabidopsis* seedlings with *T. atroviride* promotes differentiation of root architecture and an increase in biomass that correlates with the increased activity of

MPK6. Whereas *mpk6* mutants showed enhanced growth inhibition of primary roots compared to the wild-type plant, as well as a minor increase of lateral root number. Together, this information contributes to the understanding of molecular mechanisms by which *Trichoderma* promotes plant growth; however, the molecular bases of such interactions are still unclear.

Trichoderma spp. effector-like proteins

As we have seen so far, *Trichoderma* colonizes the plants roots, whose process starts with the penetration of physical barriers, resistance to the antimicrobial compounds, recognition, and its establishment into the roots (Hermosa et al., 2012). A growing number of evidences have shown that during the *Trichoderma*–plant interaction, a molecular cross-talk, mediated by molecules produced by both, is orchestrated (Djonovick et al., 2006; Salas-Marina et al., 2011, 2015). Several studies in plant–microbe interaction are referred to secreted proteins by beneficial microbes or plant pathogens called effectors, whose main function is the manipulation of plant immune responses, or may have other functions such as self-defense or liberation of nutrients from host tissues (Snelders et al., 2018). Current knowledge on fungal effectors has arisen mainly from research on biotrophic, hemibiotrophic, and necrotrophic fungal and oomycetes phytopathogens that have a limited host range (Rafiqi et al., 2013; Wang et al., 2014; McGowan and Fitzpatrick, 2017). Effector proteins are defined as stable small-secreted proteins that manipulate host cell structure and function thereby facilitating the infection, host colonization, and/or triggering defense responses (Wang and Wang, 2018; Kamoun, 2006). Furthermore, small soluble cysteine-rich secreted proteins (SSCP) (effector-like proteins) by *Trichoderma* are responsible of mediating the early stage of penetration by suppressing plant defense, which is crucial to succeed at the beginning of root colonization (Mendoza-Mendoza et al., 2017). Consequently, a successful establishment of plant–*Trichoderma* interactions probably implies the production of effector-like proteins. Effector-like proteins are exported or translocated into eukaryotic host cells. So far, putative effector-like proteins from *Trichoderma* have been identified by bioinformatic tools as well as experimentally during plant–*Trichoderma* interaction (Table 1.1) (Guzmán-Guzmán et al., 2017; Mendoza-Mendoza et al., 2017; Nogueira-Lopez et al., 2018; González-López et al., unpublished data).

Table 1.1 shows the main families of effector-like proteins predicted for *T. atroviride*, *Trichoderma reesei*, and *T. virens* by using a bioinformatic approach. Recently, *T. virens* was used to identify putative effector-like proteins through secretome analysis during their interaction with *Zea mays* plants (Nogueira-Lopez et al., 2018). In our group, a *T. atroviride*–*Arabidopsis* secretome analysis was performed using a semihydroponic system, identifying more than 1000 secreted proteins of the fungus. In addition, a bioinformatic analysis showed that some of these proteins contain characteristics common to already identified fungal effector-like proteins (González-López et al., unpublished data). The set of effector-like candidates include LysM repeats proteins, thioredoxins, hydrophobins, cerato-platanins, serine proteases, metalloproteases, and other uncharacterized proteins, most of which were previously identified by other research groups (Table 1.1).

TABLE 1.1 Effector-like proteins identified in *Trichoderma atroviride*, *Trichoderma reesei*, and *Trichoderma virens* spp. using experimental or bioinformatic approaches.

	Specie							
	T. atroviride			***T. reesei***		***T. virens***		
Main effector families/ References	Guzman-Guzman et al. (2017)	Mendoza-Mendoza et al. (2017)	Gonzalez-Lopez et. al., unpublished data	Guzman-Guzman et al. (2017)	Mendoza-Mendoza et al. (2017)	Guzman-Guzman et al. (2017)	Mendoza-Mendoza et al. (2017)	Nogueira-Lopez et al. (2018)
LysM Repeats	6	1	2	2	0	9	0	0
Thioredoxins	4	0	2	3	0	4	0	3
CFEM domain	12	1	1	10	2	10	1	1
Cerato-platanins	4	3	3	3	2	4	4	2
Serine-proteases	20	0	0	11	0	16	0	0
Hydrophobins	9	11	4	5	5	7	9	0
Metalloproteases	2	0	0	2	0	2	0	0
Other families	25	22	132	23	14	28	46	1
Uncharacterized	4	68	38	4	47	4	58	0
Approach used	Predicted using bioinformatic tools	Predicted using EffectorP	Secreted in interaction with *Arabidopsis thaliana*	Predicted using bioinformatic tools	Predicted using EffectorP	Predicted using bioinformatic tools	Predicted using EffectorP	Secreted in maize roots apoplast

SSCPs comprise the largest group of proteins secreted by *Trichoderma* spp. *T. reesei*, *T. atroviride*, and *T. virens* containing around 173 SSCPs, which are predicted to act as effectors by interfering the plant defense systems to establish a beneficial relationship (Nogueira-Lopez et al., 2018). *Trichoderma* SSCPs are usually ≤300 amino acids long and may contain four or more cysteine residues. Sequence similarity and phylogenetic analyses led to subdivide them into four groups: (i) elicitor-like proteins; (ii) hydrophobins and hydrophobin-like proteins; (iii) proteins with similarity to MRSP1 (MAP kinase repressed secreted protein 1), a 16-kDa protein bearing the conserved four-cysteine pattern C-X29-C[P/G]C-X31-C, strongly overexpressed in a delta-*tmk1* (a MAPK) mutant of *T. virens*; and (iv) SSCPs lacking a functional category (Druzhinina et al., 2012).

The elicitor-like proteins' group includes the cerato-platanin family of proteins (CPPs), which have been recently studied with some detail. CPPs contain four conserved cysteines that form two disulfide bridges and are abundantly produced by organisms with a variety of lifestyles including phytopathogens, endophytes, saprotrophs, nematophagous, and PGPF (Frischmann et al., 2013; Gaderer et al., 2014; Pazzagli et al., 2014). For instance, CCPs include the phytotoxin cerato-platanin (CP) of the phytopathogen *Ceratocystis platani*. CP orthologous are also found in other pathogens and beneficial fungi. In this concern, Sm1 (Small protein 1) from *T. virens* and its orthologous Epl1 (eliciting plant response-like) from *T. atroviride* trigger the productions of reactive oxygen species and the expression of pathogen-related genes in maize, cotton, and tomato (Djonović et al., 2006; Seidl et al., 2006; Salas-Marina et al., 2015).

Hydrophobins are small cysteine-rich proteins produced by filamentous fungi with a molecular weight ranging from 7 to 15 kDa (Piscitelli et al., 2017); cysteine residues are implicated in the formation of four intramolecular disulfide bonds that provide a rigid framework that restricts the mobility of the polypeptide chain (Lo et al., 2014; Schor et al., 2016). These extracellular proteins are known for their ability to assemble spontaneously into amphipathic monolayers at hydrophobic−hydrophilic interfaces (Wu et al., 2017). According to the cysteine spacing pattern and the hydropathy patterns, hydrophobins are divided in class I and II (Hungund et al., 2016; Pham et al., 2016, 2018; Schor et al., 2016; Piscitelli et al., 2017). Class I hydrophobins show low conservation at amino acid sequence level and are commonly located on the surface of fungal spores and form a mosaic of rodlike structures known as rodlets (Hungund et al., 2016; Pham et al., 2016, 2018; Schor et al., 2016; Piscitelli et al., 2017). Contrastingly, class II hydrophobins show higher sequence conservation at amino acid sequence level and instead of assembling into rodlets, these proteins lack the fibrillary rodlet morphology and can be solubilized with organic solvents and detergents (Hungund et al., 2016; Schor et al., 2016).

Fungi of the *Trichoderma* genus produce a wide range of hydrophobins and the roles of some of them have been established. For instance, HYTLO1, a class II hydrophobin of *Trichoderma longibrachiatum*, has a role in plant growth promotion and acts as MAMP by eliciting the plant defense response (Ruocco et al., 2015). The class II hydrophobin HFB70 is involved in protecting *T. virens* against a diversity of stress factors and likely participates in the establishment of the fungus in wetlands or other conditions related to high humidity (Przylucka et al., 2017). QID74, a hydrophobin-like protein from *T. harzianum*, is involved in fungal root adherence, cell wall protection, and changes in tomato and cucumber root architecture (Samolski et al., 2012). TVHYDII1, a class II hydrophobin family member, participates in

the antagonistic activity of *T. virens* against the root phytopathogen *Rhizoctonia solani* and favors *Arabidopsis* root colonization (Guzman-Guzman et al., 2017).

An important question to solve in plant–*Trichoderma* interaction research field is how the fungus is recognized as "a friend" or how it avoids the plant defense system. Although significant progress has been made on the identification of effector-like proteins in different *Trichoderma* species, the primary function of these proteins in the *Trichoderma*–plant interaction has not yet well elucidated. Further studies are needed to fully understand the molecular mechanisms by which effector-like proteins from *Trichoderma* species participate in plant root colonization, and further on the modulation of plant physiology to establish a symbiotic relationship.

Modulation of plant systemic resistances and the protection conferred by *Trichoderma*

Because of sessile nature of plants and their inability to escape from specific environmental conditions, stresses, or predation, they have evolved sophisticated mechanisms to counteract them and survive. The first important signal to generate a response by the plant against the pathogen is the MAMP or PAMP recognition by the plant receptors. These receptors initiate a signal transduction to induce the different pathways of systemic resistance in plants, including strengthening of the cell wall, production of phytoalexins, ethylene biosynthesis, PR protein genes expression, and the triggering of the HR (Morel and Dangl, 1997; Harman et al., 2012). However, pathogens use different strategies to succeed in their attack and progression of the host disease. Nevertheless, not all microorganisms represent a potential danger to the plant. On the contrary, some of them, such as the filamentous fungi of the genus *Trichoderma* spp., offer multiple benefits, which include the protection to plants against biotic and abiotic stress.

Biotic stress and induction of systemic disease resistances

Studies of the three-way relationship established between *Trichoderma*, the plant, and the pathogen are aimed to unravel the mechanisms involved in recognition and the cross-talk used to maintain the beneficial association between *Trichoderma* and the plant, as well as the elimination of the pathogen. To identify and deeply understand the molecular factors involved in this complex tripartite interaction, several strategies have been used, including genomics, proteomics, and metabolomics (Vinale et al., 2012).

Various plants, both mono- and dicotyledonous species, like cotton, bean, tomato, pepper, tobacco, lettuce, cucumber, maize, and rice show increased resistance to pathogen attack when pretreated with *Trichoderma* (Harman et al., 2004b). During colonization of plant roots by *Trichoderma*, a phenomenon called "priming" in plants is established. Priming is an adaptive strategy that improves the defensive capacity of plants to pathogen attack, root colonization by beneficial microbes, chewing by insects, as well as the additions of chemicals and abiotic signals. After a subsequent attack, the plant responds faster and/or stronger and results in increased resistance and/or stress tolerance (Conrath et al., 2006). The treatment of *A. thaliana* with *Trichoderma hamatum* T382 primes the plant defense system,

and the subsequent inoculation with *B. cinerea* led to an accelerated and increased activation of the plant's defense response against the pathogen (Mathys et al., 2012). These effects provoked by *Trichoderma* have been also observed for tomato, lettuce, pepper, bean, and tobacco. Given the spatial separation of both microorganisms (root and leaves), this effect was attributed to the induction of systemic resistance by *T. harzianum* T39 (De Meyer et al., 1998). The protective effect generated by *Trichoderma* against *Colletotrichum orbiculare* is not affected if mycelium or mycelium-free cultures filtrates (MFCFs) is applied to cucumber plants. Application of *Trichoderma* MFCF to cucumber plants led to the lignification at the pathogen penetration points, and an increase in superoxide, suggesting the presence of elicitors in the MFCF that induce the plant disease systemic resistance (Koike et al., 2001). Different strains of *Trichoderma* can also induce protective effects against different pathogens. For instances, root colonization of cotton plants with *T. virens* G-6 and G-11 reduced the damage caused by *R. solani*, whereas the inoculation with *T. asperellum* T-203 reduces the damage caused by *P. syringae* pv. *Lachrymans*. In the latter case, the protective effect was associated with the production of antimicrobial compounds by T-203 that promotes the reduction of pathogenic bacterial cells in leaves (Yedidia et al., 2003). *Trichoderma* also protects plants against viral pathogens. The colonization of tomato roots by *T. harzianum* T-22 reduces the viral symptoms caused by cucumber mosaic virus (CMV). This effect has been associated with the inhibition of transcription of RNA-dependent RNA polymerase of tomato, as well as the role of ROS as the second messenger in the antiviral response. In addition, a significant increase of the SA, JA/ET content in plants treated with T22 and CMV occurs. These suggest that systemic resistance mediated by phytohormones SA and JA/ET leads to the reduction of plant damage by the virus (Vitti et al., 2015, 2016). However, plant priming is not a general phenomenon. For instance, tomato lines inoculated with two different *Trichoderma* strains do not show a phenotype or the effect was detrimental to the plant; therefore, the beneficial effect of *Trichoderma* is also modulated for the plant genotype (Tucci et al., 2011).

The protective effects conferred by *Trichoderma* are associated with the induction of systemic disease resistance through the SA and JA/ET pathways. Many studies have shown that *Trichoderma* spp. induce the expression of genes related to plant defense, such as *PR1-b* and osmotin (*PR-5*) in tobacco. The expression of the two *PR* genes and the accumulation of their products were mimicked by a combination of SA and Metil JA (Chang et al., 1997). In the model plant *Arabidopsis,* the expression of *PR-1a*, *PR-2*, *PDF1.2* (Plant Defensin), *LOX-2* (lipoxygenase-2), *PAD3* (camalexin biosynthesis—related gene), and WRKY transcription factors, which are related to SA and JA/ET systemic resistant pathway (*AtWRKY54* and *AtWRKY8,* respectively), is triggered by the presence of *Trichoderma* (Sala-Marina et al., 2011; Sáenz-Mata et al., 2014). Based on these results, arises the questions: what are the molecules that induce the systemic resistance and which is the nature of them? To date, multiple putative *Trichoderma* MAMPs are known. The EIX is a potent elicitor of the HR in tomato and tobacco cultivars, which is independent of its xylanase activity. The epitope of EIX protein that cause the HR consists of TKLGE pentapeptide that is part of an exposed β-strand of the protein (Rotblat et al., 2002). The Sm1 and Epl1 proteins from *T. virens* and *T. atroviride* were purified and characterized. Sm1 proteins accumulate in *Trichoderma* hyphae during the colonization of the roots in cotton and corn seedlings, triggering local and systemic resistance (Djonovic et al., 2007; Seidl et al., 2006). A 36-amino acid motif of the N-terminal carbohydrate-binding module family 1 domain (CBD) swollenin stimulates local defense

responses in cucumber roots and leaves, conferring protection against *B. cinerea* and *P. syringae pv lachrymans* infection. This indicates that the plant might recognize the CBD domain of swollenin as a *Trichoderma* MAMP (Brotman et al., 2008).

Peptaibols are SMs with antimicrobial activity secreted by *Trichoderma,* which are also classified as potential MAMPs. These linear peptides of 5−20 amino acids in length are products of nonribosomal peptide synthetase (NRPS). The disruption of *tex1* gene that codes for an NRPS in *T. virens* causes the loss of all peptaibols forms of 18 residues. The absence of *tex1* disables *Trichoderma* to trigger an effective defense response in cucumber plants. Two synthetic 18-amino acid peptaibol isoforms (TvBI and TvBII) from Gv29-8 induce systemic protection in cucumber, against pathogenic bacteria by the production of antimicrobial compounds in cotyledons that correlates with the upregulation of hydroxyperoxide lyase (hpl), phenylalanine ammonia lyase (pal1), and peroxidases genes (Viterbo et al., 2007).

Salas-Marina et al. (2011) described by first time that *T. atroviride* induces locally and systemically an overlapped expression of SA and JA/ET pathways defense-related genes (Fig. 1.1). The induction of such genes in *Arabidopsis* confers systemic disease resistance against the necrotrophic and hemibiotrophic foliar pathogens *B. cinerea* and *Pst*DC3000 (Fig. 1.1) (Salas-Marina et al., 2011). A similar expression pattern of SA and JA/ET induced by *T. atroviride* and *T. virens* was observed in tomato. *epl1* and *sm1* overexpressing or knockout strains showed that Epl1 and Sm1 proteins play a minor role in the induction of *SlPR-5.* On the other hand, *epl1* and *sm1* result necessary to induce *SlCEVI16* and *Slα−DOX1* genes, which code for a peroxidase and an α-dioxygenase, respectively. Furthermore, Epl1 plays a major role in the induction of disease resistance in tomato against necrotrophic, but a minor role against hemibiotrophic pathogens, whereas Sm1 seems to play the opposite role (Salas-Marina et al., 2015). Interestingly, in *T. atroviride* the *hda-2* gene (histone deacetylase-2) is necessary to induce protection against the biotrophic and necrotrophic pathogens *Pst*DC3000 and *B. cinerea*. Moreover, Δ*hda-2* strain is affected in its ability to induced *PR-1a* and *PDF1.2* (SA and JA/ET markers genes, respectively) by direct contact or through its VOCs compared to the wild type (Estrada-Rivera et al., 2019).

The beneficial effect of *Trichoderma* on the induction of systemic resistance is not limited to foliar pathogens. Abdelrahman et al. (2016) showed a reduction in *Fusarium* basal rot disease symptom in onion plants pretreated with *T. longibrachiatum* compared with onion plants untreated with *Trichoderma* (Abdelrahman et al., 2016). The authors suggested the involvement of onion's metabolites in the response against *Fusarium*. The authors determined an increase in aldopentose monosaccharides and ketonic monosaccharides (including xylose, arabinose, and fructose, respectively) in *T. longibrachiatum* pretreated plants and challenged with *Fusarium*, compared with untreated plants with *Trichoderma* (Abdelrahman et al., 2016). Moreover, *T. harzianum* isolate Th58 effectively antagonizes *Fusarium oxysporum* by considerably reducing disease incidence. Inoculation of cucumber seedlings with Th58 suppresses ROS over accumulation caused by *Fusarium* and improved root cell viability. Also, the expression levels of cell cycle−related genes such as *CDKA*, *CDKB*, *CycA*, *CycB*, *CycD3;1* and *CycD3;2* in Th58 preinoculated cucumber seedlings were upregulated compared with those infected only with *F. oxysporum* (Chen et al., 2017). The cellulase Thph1 of *T. harzianum* Th22 activates the expression of defense-related genes as well as genes involved in the biosynthesis of benzoxazinoid 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA, a SM) in maize roots, inhibiting the growth of *Fusarium graminearum* (Saravanakumar et al., 2018a).

The insect pests also cause biotic stress. The resistance against insects is through metabolic pathway involving the production of defense-related compounds, either directly against herbivore insects or indirectly by the attraction of herbivores natural enemies. For instance, *Trichoderma* T22 induces a primed estate in tomato plants against the aphid *Macrosiphum euphorbiae* by enhancing the production of VOCs like methyl-salicylate and Z-3-exen-1-ol that attract to *Aphidius ervi*, a small wasp that parasitizes the aphid (Coppola et al., 2017).

The terpene ergosterol that has a role as structural component of the fungal cell membrane also plays a role as elicitor of the systemic resistance in plants (Spanova et al., 2012). The trichothecenes are sesquiterpenoid compounds produced by filamentous fungi belonging to the genera *Fusarium, Stachybotrys*, and *Trichoderma*, among others (McCormick et al., 2011). The trichothecene, trichodermin produced by *Trichoderma brevicompactum*, is highly toxic for plants, whereas *harzianum* A from *Trichoderma arundinaceum* does not show any phytotoxic activity (Nielsen et al., 2005; Tijerino et al., 2011; Malmierca et al., 2012). A *T. harzianum* mutant in *erg1* gene, which codes for a squalene epoxidase, a key enzyme in the biosynthesis of triterpene derivatives such as ergosterol, accumulate lower level of ergosterol but higher level of squalene (Cardoza et al., 2006). The squealene an ergosterol precursor also acts as an important elicitor molecule of the expression of tomato defense−related genes (Malmierca et al., 2015). A number of VOCs that have been studied extensively in bacteria also elicit the systemic disease resistance (Ryu et al., 2004), but little is known in *Trichoderma*. Estrada-Rivera et al. (2019) showed that *T. atroviride* WT VOCs increases the expression levels of SA- and JA/ET-related genes in *Arabidopsis*; however, a mutant strain in a histone deacetylase (Δ*hda-2*) resulted in an altered production of VOCs, showing also an incapability to trigger an adequately SA and JA/ET resistances pathway in *Arabidopsis* plants. This suggests that *Trichoderma* needs a functional HDA-2 to allow the adequate production of VOCs, to adequately induce the plant systemic resistance. In this regard, HDA-2 is a positive regulator of MAPMs that detects the plant to induce systemic resistance. For instance, the expression of *Trichoderma* genes involved in the communication with the plant such as *epl-1*, *epl-2*, and *pbs-1* (cerato-platanin elicitor protein -1, -2, and the peptaibol synthetase PBS-1, respectively) were affected by the absence of HDA-2 in *T. atroviride*. Moreover, genes that code for transporters to tolerate the plant antimicrobial compounds, such as *abc-2* (ABC transporter), are downregulated in Δ*hda-2*. This may explain the poor colonization of *Arabidopsis* roots by Δ*hda-2*. Whereas genes involved in secondary metabolism such as *ctf-1* and *pbs-2* are upregulated in Δ*hda-2*, this may explain the overproduction of VOCs in Δ*hda-2* (Estrada-Rivera et al., 2019).

So far, we have seen that *Trichoderma* induces the resistances pathway triggered by SA and JA/ET phytohormones (Salas-Marina et al., 2011). However, it seems that the SA pathway plays a more important role for the establishment of *Trichoderma*−plant interaction. In this regard, *T. harzianum* is able to penetrate the vascular tissue of the *Arabidopsis* SA-deficient mutant *sid2*, due to the absence of callose deposition in such mutant. Also, the high induction of the JA-related gene *LOX-1* in *sid*2 mutant is consistent with its impairment in SA biosynthesis. In conclusion, the plant requires the induction of SA pathway to avoid colonization of the vascular tissue, and invasion to the entire plant, as well as to prevent the death of the plant (Alonso-Ramírez et al., 2014).

The induction of defense-related genes in plants by MAMPs requires the transduction of signals through membrane receptors that recognized MAMPs, where mitogen-activated

protein kinases (MAPKs) are involved. In this sense, *T. asperellum* induces the plant systemic resistance through a mechanism that employs JA/ET pathway resulting in the activation of a *Trichoderma*-induced protein kinase (TIPK) gene in cucumber, the orthologous pathway to *A. thaliana* MAPK3 that plays a role in the transcription factor activation during plant–microorganism interaction from the JA/ET pathway (Shoresh et al., 2006). Moscatiello et al. (2018) showed the perception of *T. longibrachiatum* hydrophobin HYTLO1 by *Lotus japonicus* plants. *L. japonicus* responded to the application of HYTLO1 through a rapid cytosolic Ca^{2+} increase and the induction of the defense-related genes, including the mitogen-activated protein kinase 3 (*MPK3*), the transcription factor *WRKY33*, and cytochrome P450. Furthermore, they showed that the rapid cytosolic Ca^{2+} increase in *L. japonicus* cells is mediated by nicotinic acid adenine dinucleotide phosphate (NAADP), a potent Ca^{2+} mobilizing messenger. This provides evidence of the participation of NAADP-gated Ca^{2+} release as a signaling pathway triggered by a biotic stimulus (Moscatiello et al., 2018). Saravanakumar et al. (2018b) described the interaction of the maize root receptors *ZmATG3* and *ZmGLP* with the hydrolases Thph1 and Thph2 from *T. harzianum*. The autophagocytosis-associated protein *ZmATG3* interacts directly with cellulase Thph1, whereas Thph2 interacts with the germin-like protein *ZmGLP* in maize roots, initiating a signaling cascade that result in the induction of SA- and JA/ET-related genes as PR-10, PAL1, and LIPASE in maize (Fig. 1.1) (Saravanakumar et al., 2018b). The *ΔThph1* and *ΔThph2* mutants failed in the induction of such defense-related genes (Saravanakumar et al., 2018a). This suggests that such receptors recognize the *Trichoderma* MAMPs to trigger a response to the beneficial fungus.

Abiotic stress

Abiotic stresses such as salinity, drought, and heavy metals negatively affect the plant growth, development, and crop yields. Abscisic acid (ABA) is an isoprenoid phytohormone involved in the response to environmental stresses, with multiple roles during the plant development and vegetative growth (Verslues and Zhu, 2005). In normal physiological states, the levels of reactive oxygen species (ROS) in the plant are maintained through the activation of the enzymatic antioxidant systems. Nevertheless, under environmental stresses, this balance is disrupted in the plant cells, resulting in an oxidative damage (Hernández and Almansa, 2002). In addition, salinity stress provokes a physiological alteration in the plant, such as an increase in the ROS production and ABA accumulation, which promotes stomatal closure to avoid water loss (Misra and Saxena, 2009). Furthermore, plants have developed protective enzymatic systems to neutralize the oxidative stress caused by salinity, which comprises ROS scavenging enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and glutathione reductase (Ma et al., 2012).

One of the most common methods for alleviating salt stress in plants is the application of oligochitosan (jasmonic acid, nitric oxide, calcium nitrate), chitooligosaccharides (gibberellic acid and calcium chloride), and ascorbic acid (Khan et al., 2010; Younis et al., 2010; Ma et al., 2012; Qiu et al., 2014; Tian et al., 2015; Zou et al., 2014). Application of PGPB and PGPF to induce plant abiotic resistance has emerged as a new approach to alleviate plant stresses. For instance, *T. harzianum*–treated seeds germinate faster and uniformly under osmotic, salinity, or temperature stresses compared to untreated seeds. Furthermore *T. harzianum*–treated seeds accumulate reduced levels of lipid peroxides (which are in part related to a shift

in the balance between the production and scavenging of reactive oxygen species) under osmotic stress compared to *Trichoderma* untreated seeds. On the other hand, the application of glutathione (antioxidant), or *T. harzianum*, generates a similar positive effect on seed germination (Mastouri et al., 2010). Wheat plants inoculated with *T. longibrachiatum* increase their chlorophyll and water content in roots and leaves. The treatment of wheat seedlings with *T. longibrachiatum* and exposed to salt stress enhanced the antioxidant activity of SOD, POD, and CAT enzymes compared to wheat-untreated plants with *T. longibrachiatum* but submitted to salinity, which parallels with the upregulation of *SOD*, *POD*, and *CAT* genes (Zhang et al., 2016a). The application of NaCl to *Brassica juncea* seedlings decreases the plant height, root length, and dry weight, whereas those plants treated with *T. harzianum* show the opposite phenotype by increasing the shoot and root length, as well as dry weight compared to control plants. These effects in plants paralleled with a reduction in the accumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (a lipid peroxidation stimulant) (Ashraf et al., 2010). The NaCl stress acts suppressing the uptake of S, Mn, Mg, Ca, and K in roots and shoots; however, the treatment of plants with *Trichoderma* restores the nutrients uptake (Ahmad et al., 2015). On the other hand, cocultivation of *Arabidopsis* seedlings with *Trichoderma* improves salinity tolerance through the activation of the auxin pathway, a relevant pathway for induction of lateral roots and Na elimination through REs (Contreras-Cornejo et al., 2014a). *Arabidopsis* seedlings treated with *T. virens* and *T. atroviride* close their stomata and avoid loss of water compared to untreated plants. However, *abi1-1* and *abi2-1* (ABA-insensitive) mutant lines do not close their stomata in response to exogenous ABA or drought stress (Roelfsema and Prins, 1995; Pei et al., 1997). Also *T. virens* and *T. atroviride* induced the ABA inducible marker *abi4::uidA* as well as the synthesis of ABA in plants under standard or saline growth conditions (Contreras-Cornejo et al., 2015).

On the other hand, drought stress reduces the chlorophyll and carotenoid contents and severe membrane damage in maize plants; however, inoculation of maize with *T. atroviride* ID20G increases fresh and dry weight of maize roots under drought stress. *T. atroviride* ID20G also prevents an increase in lipid peroxidation and reverts the changes caused by drought in pigment contents and photosystem efficiency. Moreover, antioxidant enzyme activity is induced by *Trichoderma*, which parallels with a diminishment of H_2O_2 content in response to drought stress, suggesting that root colonization by this fungus may help in reducing detrimental effects of drought in maize seedlings (Guler et al., 2016). The VOCs of *Trichoderma* spp. have been involved in the resistance to salinity stress. Treatment of *Arabidopsis* with *Trichoderma koningii*, *T. asperellum, T. atroviride, T. harzianum, Trichoderma koningiopsis, T. virens, and Trichoderma viridescens* VOCs promote plant growth, even under salinity conditions. In addition, plants exposed to *T. koningii* VOCs showed less accumulation of H_2O_2 under NaCl compared to control plants (Jalali et al., 2017).

Endophytes microorganisms can also induce tolerance to abiotic stress, such as drought. The endophyte *T. hamatum* provokes a delayed response to drought, paralleling with the expression of drought-responsive genes like rubisco small subunit, senescence-associated protein, or pathogenesis-related protein 5 (PR-5), provoking a retardation in net photosynthesis and stomatal conductance in *Theobroma cacao* (Bae et al., 2009). The rice-endophyte *T. harzianum* TH-56 induces the expression of aquaporin, dehydrin, and malondialdehyde genes, and plants show higher accumulation of SOD, increased plant height, and total dry biomass (Pandey et al., 2016). Interestingly, the opposite effect is observed after the

application of the fertilizer (NPK 14:16:18) to tomato plants, previously inoculated with *T. harzianum*. A misregulation of the phytohormones network and an increase in sensitivity to salt stress by the plant were determined (Rubio et al., 2017).

Furthermore, human activities have considerably increased the amounts of Cu (copper) in agricultural soils, reaching concentrations between 200 and 1000 mg/kg (Mirlean et al., 2007; Pietrzak and Uren, 2011). When Cu concentrations exceed 50 mg/kg in soil, plants and microorganisms are subjected to its toxic effects (Borkow and Gabbay, 2005; Nagajyoti et al., 2010). Cu phytotoxicity reduces crop yield and causes poor seed germination, chlorosis, necrosis, stunting of leaves, and reduced root growth (Kumar et al., 2008; Adrees et al., 2015; Ferreira et al., 2015). In addition, redox cycling between Cu^{2+} and Cu^{1+} catalyzes the production of highly reactive hydroxyl radicals, which subsequently damage lipids, proteins, DNA, and other biomolecules in plants and microorganisms (Borkow and Gabbay, 2005; Valko et al., 2005; Nagajyoti et al., 2010; Sytar et al., 2013). *Trichoderma* has emerged as an environmentally friendly microorganism, able to modulate tolerance to stress provoked by heavy metals in plants. The excess of Cu (100 and 250 μM $CuSO_4$) results toxic to *T. asperellum*, whereas lower Cu concentrations (50 μM $CuSO_4$) do not affect the fungus growth. When onion plants were inoculated with *T. asperellum* prior to Cu exposure (50 μM), Cu accumulation and translocation in tissues were reduced and Cu toxicity was ameliorated, which was reflected in an increased growth, high chlorophyll content, and the reduction of malondialdehyde (Vargas et al., 2017). Arsenic (As) is another heavy metal that causes plant stress. Arsenic can neither be eliminated nor destroyed from environment; however, microbial biotransformation is one way to turn As into a less/nontoxic form. It is known that fungi methylate inorganic As (iAs) to a less toxic organic forms such as monomethyl arsenic acid, dimethyl arsonic acid, and trimethylarsine oxide, thereby reducing As toxicity in the chickpea plants (Tripathi et al., 2015; Verma et al., 2016). Some of the effects of iAs in plants includes inhibition of energy-linked functions of the mitochondria, chlorophyll biosynthesis, and ROS generation (Kumar et al., 2015; Mishra et al., 2014). *Trichoderma* alleviates phytotoxicity in lettuce and chickpea plants irrigated with As-contaminated water (Caporale et al., 2014; Tripathi et al., 2015). Tripathi and collaborators discovered and used two *Trichoderma* strains As tolerants, to study the role of *Trichoderma* to alleviate As-induced toxicity in chickpea. Chickpea plants growing in an arsenate soil and then inoculated with M-35 (As tolerant) and PPLF-28 (As sensitive) *Trichoderma* strains, similar levels of As were found in both *Trichoderma* treatments. However, a difference was observed in the levels of organic and inorganic As (iAs), which correlates with an enhanced plant-growth promotion and nutrient content in plants by M-35 compared to PPLF-28. A downregulation of plant abiotic stress responsive genes, myo-inositol phosphate synthase (*MIPS*), polygalacturonase inhibiting protein (*PGIP*), and 3β glucosidase (*CGG*), was observed in plants treated As with and a tolerant *Trichoderma* strain (M-35), whereas those plants treated with *Trichoderma* sensitive (PPLF-28) plus As or those plants treated only with As, an upregulation was observed (Tripathi et al., 2017). All evidences support that the different *Trichoderma* strains increase seedling vigor and ameliorates stress by inducing physiological protection in plants against pathogens, oxidative damage, salinity, drought, and heavy metal stress. Further studies are needed to fully understand the molecular mechanisms by which *Trichoderma* induces priming against biotic and abiotic stresses in plants.

Emerging mechanisms in *Trichoderma*–plant interaction

Chromatin structure and modifications

The readout of the genetic information is regulated by chromatin modifications, which induce changes in gene expression in response to internal or external cues during growth, differentiation, development, metabolic processes, and abiotic and biotic stresses (Brosch et al., 2008). Genomic DNA in the eukaryotic cell nucleus needs to be compacted in a highly organized structure termed chromatin. The nucleosome is the fundamental unit of chromatin and consists of ≈147 base pairs of superhelical DNA wrapped around four core histones (H3, H4, H2A, and H2B) (Luger, 2003). Nucleosomes are separated by the linker histone H1, which are substantially less conserved between organisms (Baxevanis and Landsman, 1996). The core histones are predominantly globular except for their N-terminal "tails," which are unstructured (Kouzarides, 2007). Histones can undergo posttranscriptional modifications that alter their interaction with DNA and nuclear proteins (Strahl and Allis, 2000). There are at least eight distinct types of modifications found on histones such as acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP ribosylation, deimination, and proline isomerization (Kouzarides, 2007). Histone modifications may influence the higher-order chromatin structure by affecting the contact between different histones in adjacent nucleosomes or the interaction of histones with DNA resulting in chromatin structural rearranges (Kouzarides, 2007). In the *Trichoderma* spp. genome, the four core histones, H2A, H2B, H3 and H4, and the linker histone H1 have been found, thereby confirming the existence of the basic components of the nucleosomes (Schmoll et al., 2016). Unlike histone chemical modifications, chromatin remodeling complexes use the energy of ATP hydrolysis to change the packaging state of chromatin by moving, ejecting, or restructuring the nucleosome (Becker and Hörz, 2002; Clapier and Cairns, 2009). There are currently four different families of chromatin remodeling complexes which comprises SWI/SNF (switching defective/sucrose nonfermenting), ISWI (imitation switch), CHD (chromodomain, helicase, DNA binding), and the INO80 (inositol requiring 80) family remodelers (Lusser and Kadonaga, 2003; Clapier and Cairns, 2009). In *Trichoderma* spp. genomes have been predicted from 22 to 28 proteins related to the SWI/SNF ATPase/helicase domain proteins. Interestingly, the Rad 5/16-like subfamily are the most represented group of predicted proteins in the *Trichoderma* spp. genomes, which belong to the Snf2 family (Schmoll et al., 2016).

Secondary metabolism and histone methyltransferases

Trichoderma spp. are SMs producers. SMs are small molecules not necessary for growth, development, or reproduction. These compounds have been categorized into three major classes: (i) volatile antibiotics and isocyanide derivatives; (ii) water-soluble compounds, and (iii) peptaibols (Sivasithamparam and Ghisalberti, 1998). Production of SMs by *Trichoderma* depends of the strain, environmental conditions, and their production may require specific triggering stimuli (Vinale et al., 2009). *Trichoderma* SMs have been involved in autoregulatory processes, plant-growth promotion mechanisms, plant defense responses, and as inhibitory compounds against bacteria, yeasts, and fungi (Reino et al., 2008; Vinale et al., 2012; Zeilinger et al., 2016). Genes involved in SMs biosynthesis are typically arrayed

in gene clusters such as NRPSs, polyketide synthases (PKSs), or terpene synthases/cyclases (Mukherjee et al., 2012a, 2013; Bansal and Mukherjee, 2016). The production of SMs is tightly regulated in fungi and some of the biosynthetic clusters have been proved to be under the control of chromatin modifications such as histone methylations. Methylation can occur on either lysine (K) or arginine (R) residues of histones H3 or H4 (Bannister and Kouzarides, 2005). Histone methyltransferases catalyze the transfer of methyl groups from the S-adenosylmethionine (SAM) to either the ε-NH_2 group of lysine or the ω-or δ-NH_2 of arginine residues (Zhang and Reinberg, 2001). Functionally, lysine methylation has been correlated with both gene activation and silencing, depending on the modified residue and number of methylations. Over the years, the link between secondary metabolism and chromatin modifications has been extensively studied in *Aspergillus* spp. The first analysis resulted in the identification of the global regulator of secondary metabolism, LaeA a putative SAM-dependent methyltransferase. Lacking strains of *laeA* in *Aspergillus* spp. are affected in the expression of sterigmatocystin (carcinogen), penicillin (antibiotic), and lovastatin (antihypercholesterolemic agent) gene clusters (Bok and Keller, 2004). In *T. reesei*, the deletion of *lae1* causes a loss in the expression of seven cellulases, auxiliary factor for cellulose degradation, β-glucosidases, and xylanases. Conversely, enhanced expression of *lae1* resulted in significantly increased cellulase gene expression (Seiboth et al., 2012). However, high-throughput sequencing (ChIP-seq) results show that *lae1* mutants does not correlate with H3K4me2 and H3K4me3 (gene transcription) or H3K9me3 (gene silencing), suggesting that LAE1 does not affect gene expression by directly modulating H3K4 or H3K9 methylation (Seiboth et al., 2012). Although, in a further analysis, it was found that LAE1 positively regulates the expression of seven polyketide or nonribosomal peptide synthases, genes encoding ankyrin proteins, iron uptake, heterokaryon incompatibility proteins, PTH11-receptors, and oxidases/monoxygenases gene categories. Interestingly, by means of ChIP-seq with antibodies against H3K4me2 and H3K4me3, and H3K9me3 were able to identify 4089 genes bearing one or more of these methylation marks on the parental, Δ*lae1*, and the *lae1*OE (overexpression of *lae1*) strain, of which only 75 genes were LAE1 regulated (Karimi-Aghcheh et al., 2013). The Velvet global regulators are specific transcription factors, comprised by VosA (Viability of spores A), VeA, VelB (Velvet like protein B), and VelC, which play diverse roles in the *Aspergillus* spp. growth, development, and secondary metabolism (Park and Yu, 2016). It has been shown that VelB-VeA-LaeA form multimeric velvet complexes to govern development and secondary metabolism in *Aspergillus* spp. in response to light (Bayram et al., 2008). In *Trichoderma* spp., the global velvet regulators have been identified and analyzed. Deletion mutants of *vel1* in *T. reesei* were defective in secondary metabolism (did not produce gliotoxin) and mycoparasitism (did not overgrow or lyse *R. solani* and *Pythium ultimum*) (Mukherjee and Kenerley, 2010). Moreover, the biocontrol efficacy of Δ*lae1* was impaired, showing that Δ*lae1*-treated seeds sown in a pathogen-infested soil (*P. ultimum* and *R. solani)* were more susceptible to the phytopathogen attack (Mukherjee and Kenerley, 2010). A few years later, a new role for VELVET in *T. reesei* was discovered, which linked light, development, and secondary metabolism (Bazafkan et al., 2015). Interestingly, lack of *vel1* affected the regulation of the pheromone system (*hpr1*, *hpr2*, *hpp1*, and *ppg1*) in a mating type−dependent manner, suggesting a VEL1 role in communication between potential partners in the fungus (Bazafkan et al., 2015). Besides methyltransferases, recently, it has been proposed that a new family of Ras-GTPases, the Big Ras-GTPases (BRG), is involved in conidiation, development, secondary metabolism, and biocontrol in *T. virens* (Dautt-Castro et al., 2020). In this regard, TBRG-1

seems to be a negative regulator of secondary metabolism, since mycelium-free culture filtrates of *Δtbrg-1* mutants show enhanced antibiotic activity against *R. solani* and produce more gliotoxin, compared to wt (Dautt-Castro et al., 2020). Controversially, *Δtbrg-1* strain is not able to protect tomato seedlings against *R. solani*, on the contrary, *Δtbrg-1* behaves like a plant pathogen (Dautt-Castro et al., 2020).

Histone acetyltransferases in plant−*Trichoderma* interaction

The link between chromatin modifications and plant *Trichoderma* spp. interaction has been scarcely studied. However, recently, the epigenomic aspects of plant immunity and the role of chromatin modifications for plant−pathogen counterattack have become evident in the field of plant−microbe interaction. In this regard, the role of the histone deacetylase HDA-2 in *T. atroviride*, in plant growth promotion, triggering of plant defense responses, and production of SMs, has been addressed. Histone acetylation is a dynamic modification conducted by histone acetyltransferases (HATs), which involves the reversible transfer of the acetyl moiety Acetyl-CoA (acetyl coenzyme A) to the ε-amino group of lysine, resulting in the neutralization of a positive charge (Allfrey et al., 1964). Histone acetylation is mostly associated with the transcription activation. In contrast, HDACs catalyze the removal of acetyl groups from lysine residues, reestablishing the positive charge of the lysine and stabilizing the chromatin architecture (Roth et al., 2001; Bannister and Kouzarides, 2011). The reversal of acetylation correlates with transcriptional repression (Gregoretti et al., 2004). In *T. atroviride*, the histone deacetylase HDA-2 regulates growth, conidiation, blue light perception, and oxidative responses. The Δ*hda-2* strain shows low accumulation of H3K9K14ac on the promoter regions of *cat-3* and *gst-1* ROS-related genes, pointing out to an indirect regulation of HDA-2 on those genes (Osorio-concepción et al., 2017). The absence of HDA-2 impairs *T. atroviride* ability to colonize roots, and to adequately induce the defense responses in *A. thaliana*. Strikingly, the Δ*hda-2* VOCs showed an enhanced effect on lateral root stimulation of *A. thaliana* seedlings compared to the WT. The production of SMs in the Δ*hda-2*-strain was also affected, resulting in the overproduction of 6-PP.

ChIP of H3acK9/K14/K18/K23/K27 on the promoter regions of *epl-1* (plant-responsive gene) and *abc-2* (defense against toxic compounds) presented low acetylation levels, whereas *ctf-1* (synthesis of SM) showed high constitutive levels, which correlated with the level expression of these plant-responsive genes in the Δ*hda-2* strain, supporting a dual role for HDA-2 in the regulation of plant-responsive genes (Estrada-Rivera et al., 2019).

Chromatin remodelers in plant−*Trichoderma* interaction

A transcriptomic analysis of *T. virens* during its interaction with *A. thaliana* showed the repression of carbohydrate metabolism (cell wall degrading enzymes) at the beginning of the interaction, which stopped at later times with the induction of copper ion transport, suggesting the relevance of provide metals as cofactors for cell wall degrading enzymes in *T. virens*−plant interaction. RNA-Seq results showed the upregulation of *ipa-1* (increased protection of *Arabidopsis-1*), which codes for a putative chromatin remodeler/helicase-related protein. Lacking of *ipa-1* in *T. virens* does not affect conidiation, mycoparasitism, or antibiosis

against *B. cinerea*. However, *Arabidopsis*-Δ*ipa-1*-treated plants showed a higher induction of the plant defense response, compared to those treated with the wild-type strain. Plants were also more resistant to the phytopathogen *P. syringae* DC3000, suggesting that IPA-1 might act as a negative regulator of genes involved in the SA and JA/ET pathway, such as effectors or SMs (Estrada-Rivera et al., 2020).

Small RNA pathway involved in *Trichoderma*—plant interaction

During the last years, several researches have shown that small RNAs (sRNAs) and the components of the sRNAs-mediated silencing machinery play important roles in plant immunity against virus, bacteria, fungi, and plant symbionts, through mobile sRNAs (Katiyar-Agarwal and Jin, 2010; Lauressergues et al., 2012). In Eukarya, sRNAs function as regulatory elements mediating gene silencing in a sequence-specific mode by complementarity and degradation of their mRNAs targets or through the inhibition of their translation. sRNAs can regulate gene expression at transcriptional level through DNA methylation or chromatin modifications, a pathway known as RNA-directed DNA methylation (RdDM) as well. sRNAs regulate cellular growth, metabolism, maintenance of genome integrity, and responses to different types of stress (Katiyar-Agarwal and Jin, 2010). In plants, sRNAs are generated by Dicer-like proteins, which take as template double-stranded RNAs or single-stranded RNA forming hairpin structures. The *Arabidopsis* genome codes for four DCL proteins that catalyze the generation of sRNAs, ranging from 20 to 24 nucleotides in size. Upon dicing, sRNAs are loaded into *Arabidopsis* Argonaute proteins (AGO), which are part of the RNA-induced silencing complexes (RISC), leading to transcriptional gene silencing (Baulcombe, 2004; Castel and Martienssen, 2013; Chang et al., 2012). Several members of the sRNAs biogenesis machinery are involved in plant immunity, including DCL, AGO, RNA-dependent RNA polymerases (RDRs), and plant-specific DNA-dependent RNA polymerases (POL) proteins. Mutants in such genes show defects in sRNAs accumulation and impaired responses to pathogens (Seo et al., 2013). In this sense, infection of plants with the oomycete phytopathogen *Phytophthora sojae* leads to a differential accumulation of sRNAs, contributing to soybean basal resistance (Wong et al., 2014). Moreover, the release of effectors by *Phytophthora* (PsPSR1 and PsPSR2) functions as RNA silencing suppressors by interfering the plant sRNAs biogenesis machinery (Qiao et al., 2013). Furthermore, the SA—defense pathway is enhanced in *Arabidopsis* Pol V-defective mutants, whereas JA—defense pathway is downregulated (López et al., 2011). On the other hand, the expression levels of *Arabidopsis AGO2* gene is highly induced by the bacterial pathogen *Pst*DC3000. Additionally, AGO2 binds miR393b* to modulate exocytosis of the antimicrobial PR proteins. Mutants in *AGO2* are highly susceptible to *Pst*DC3000 (Zhang et al., 2011). In 2013, Weiberg and colleagues reported that the fungal pathogen *B. cinerea* employs sRNAs to modulate plant immunity, finding that *B. cinerea* sRNAs bind and kidnap the *Arabidopsis* AGO1 protein, to selectively downregulate host immunity—related genes (Weiberg et al., 2013). Since then, sRNAs have been classified as a new class of effector molecules, which modulate plant immunity. Recently, a novel plant defense strategy has been described, where plants export specific miRNAs into the fungus to silence virulence-related genes in *Verticillium dahliae*, leading to a loss of pathogenicity

(Zhang et al., 2016b). The production of sRNAs by filamentous fungi, including *Trichoderma* species, has been documented. The genome of *T. atroviride* bears two dicer (*dcr1* and *dcr2*), three argonaute (*ago1*−*ago3*), and three RNA-dependent RNA polymerases (*rdr1*−*rdr3*) homologues genes. Although analyses of mutants in such genes are affected in conidiation presenting morphological alterations, there is still research to be done on the roles of sRNAs in this kingdom (Carreras-Villaseñor et al., 2013). In particular, there are no direct evidences of the involvement of the sRNAs-silencing machinery in modulating plant immunity or plant growth promotion induced by *Trichoderma.* In our working group, we are trying to understand the role of the small RNA pathway in the establishment of the beneficial relationship *Trichoderma*−*Arabidopsis*. Until now, we have found that the inoculation of *T. atroviride* in *Arabidopsis* roots enhances the expression level of *DCL* and *AGO* genes in roots and leaves. Furthermore, *ago2*, *ago4*, *rdr2*, and *polv Arabidopsis* mutant lines are affected on the benefits conferred by *Trichoderma* (Rebolledo-Prudencio et al., under review). Moreover, we sequenced sRNAs libraries during *Arabidopsis*−*T. atroviride* interaction. Mapping of sRNAs over the *T. atroviride* genome revealed that 37 sRNAs of the fungus matched on genes of the host plant *Arabidopsis* (Fig. 1.1). Interestingly, target genes code for lytic enzymes, MAP kinases putatively involved in plant immunity, protein with domains of unknown function, disease resistance protein NBS-LRR class family, s-adenosyl-L-methionine-dependent methyltransferases superfamily proteins, among others. This indicates that *T. atroviride* could be using sRNAs as effector molecules, similarly as *B. cinerea* and *V. dahliae*, to establish a symbiotic relationship with plants. However, more research on the role of sRNAs is needed to well understand the molecular mechanisms mediated by the fungal sRNAs that allows us to better understand the molecular dialogue that leads to the establishment of this beneficial association.

Concluding remarks

In the last decade, several important works on *Trichoderma*-plant interaction had shed light on this relationship. These publications have contributed to our understanding of this complex mutualistic relationship, which implies an special cross communication to distinguish a mutualistic from a deleterious relationship. During this interaction, it is worth to highlight the processes of fungus-plant and plant-fungus recognition during root colonization, probably implying the release of effector and elicitor molecules that modulate and trigger the plant response, generating protective effects against biotic and abiotic stress, as well as the benefits conferred by the fungus such as plant growth and development stimulation. Among the most important advances is included the discovery of receptors in maize roots that recognize the effector molecules released by *Trichoderma*, which triggers the transduction of signals in the plant cells to recognize the fungus. Moreover, it has been demonstrated that the chromatin modifications in the fungus, and the small RNA-based gene silencing in plants also contributes to this mutualistic relationship. Despite the current knowledge of this beneficial interaction, there are still many unanswered questions regarding the molecules that participate in the plant-fungus recognition from both partners. Furthermore, with the findings of bidirectional cross-kingdom communication mediated by small RNAs in between fungi

and plants, arises the question whether or not *Trichoderma* or the plant uses sRNAs to modulate the expression of genes of its partner to establish a mutualistic relationship. In this sense, there would also be possible that proteins from the plant could be exerting a role as "effectors", preventing the over-colonization of plant roots by *Trichoderma*. Regarding the chromatin modifications provoked during the interaction, we also wonder if such chemical changes could be inherited from one generation to the next in plants. Although there are still so much unanswered questions, the knowledge about this beneficial interaction between *Trichoderma* and plants is increasing. This new knowledge will help to develop new strategies to find and use *Trichoderma* strains to mitigate biotic and abiotic stress in plants of commercial interest to increase their yield in the field.

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