



Trichoderma/pathogen/plant interaction in pre-harvest food security

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ABSTRACT

Large losses before crop harvesting are caused by plant pathogens, such as viruses, bacteria, oomycetes, fungi, and nematodes. Among these, fungi are the major cause of losses in agriculture worldwide. Plant pathogens are still controlled through application of agrochemicals, causing human disease and impacting environmental and food security. Biological control provides a safe alternative for the control of fungal plant pathogens, because of the ability of biocontrol agents to establish in the ecosystem. Some *Trichoderma* spp. are considered potential agents in the control of fungal plant diseases. They can interact directly with roots, increasing plant growth, resistance to diseases, and tolerance to abiotic stress. Furthermore, *Trichoderma* can directly kill fungal plant pathogens by antibiosis, as well as via myco-parasitism strategies. In this review, we will discuss the interactions between *Trichoderma*/fungal pathogens/plants during the pre-harvest of crops. In addition, we will highlight how these interactions can influence crop production and food security. Finally, we will describe the future of crop production using antimicrobial peptides, plants carrying pathogen-derived resistance, and plantibodies.

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1. Introduction

Annually, large agricultural losses occur worldwide due to the susceptibility of crops to diseases caused by plant pathogens, impacting productivity and reducing the commercial value of the product. It is estimated that 78 % is lost in fruit crops, 54 % in vegetable crops, and 32 % in cereal crops due to diseases caused by pathogens (Zhang, 2018). Plant pathogens are described as being responsible for the large-scale destruction of various types of crops worldwide, and can cause large losses in crops susceptible to diseases both in the field (pre-harvest) and post-harvest. The major groups of pathogens are viruses, bacteria, oomycetes, fungi, nematodes, and parasitic plants (Strange and Scott, 2005). Fungi are the primary cause of large losses in the world's major crops, such as rice, beans, soybeans, corn, potatoes, and wheat (Fisher et al., 2012).

The traditional means of combating plant pathogens is through the application of agrochemicals. According to the Food and Agriculture Organization (FAO), the use of pesticides by continent is 52.2 % in Asia, 29.4 % in the Americas, 14 % in Europe, 2.1 % in Africa, and 1.2 % in Oceania. Pesticide use has grown from 2000 tons of active ingredients in 1990 to 4000 tons in 2016. The application of fungicides is an efficient but expensive process, and can cause damage to human and animal health, environmental damage, the development of resistant pathogens, and the appearance of secondary pests. Furthermore, nonspecific fungicides can eliminate microorganisms already established in the soil, increasing the susceptibility of plants to soil pathogens (Heydari and Pessaraki, 2010).

An alternative to the use of fungicides is biological control, a method applied in the use of antagonistic microorganisms suppressing diseases, as well as host-specific pathogens to control weed populations. The pest-suppressing organism or host-specific pathogen is referred to more broadly as the biocontrol agent (BCA). The term “biocontrol” may be used for natural products extracted

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Abbreviations			
BCA	biocontrol agent	PTI/MTI	pathogen/microbe-triggered immunity
CWDEs	cell wall-degrading enzymes	ETI	effector-triggered immunity
CAZY	is a database of Carbohydrate-Active enZYmes (CAZymes)	RLKs	receptor-like kinases
GH	Glycosyl hydrolases	RLPs	receptor-like proteins
EST	expressed sequence tag	NB-LRR	nucleotide-binding domain leucine-rich repeat
SSH	suppression subtractive hybridization	HR	hypersensitive response
CFEM	Common in Fungal Extracellular Membrane protein	SAR	systemic acquired resistance
NPP1	Ectonucleotide pyrophosphatase/phosphodiesterase-1	PGPR	plant growth-promoting rhizobacteria
SM	secondary metabolites	PGPF	plant growth-promoting fungus
CBD	carbohydrate-binding domain	ISR	induced systemic resistance
PAMP/MAMP	pathogen/microbe-associated molecular pattern	MAMP	microbe-associated molecular pattern
		DAMPs	Damage-associated molecular patterns
		AMPs	antimicrobial peptides
		PDR	Pathogen-derived Resistance

or fermented from various sources. Such formulations may be simple or complex blends of natural ingredients, with either a specific activity or multiple effects on the host. For complex mixtures, and depending on the primary benefit provided to the host plant, these may be termed biopesticides or biofertilizers (Heydari and Pessaraki, 2010). While fungicides have only a temporary effect and usually require repeated applications during the cropping season, biological control agents are able to establish themselves in the ecosystem, reproduce, and colonize the rhizosphere, phyllosphere, and rhizoplane (Zeilinger et al., 2016). In addition, biological control strategies are highly compatible with the self-sustaining farming practices necessary for the conservation of natural resources for agriculture (Liu et al., 2008).

Over the years, several researchers have been showing the applications of fungi species in agriculture, wherein various species have the ability to alter plant metabolism by providing resistance to abiotic and biotic stress (Kumar et al., 2012). Some species of the genus *Trichoderma* are considered potential BCAs in plant disease control, being an alternative for the control of phytopathogens (Keswani et al., 2014). Furthermore, *Trichoderma* have been observed to interact directly with roots, resulting in increased plant growth potential, resistance to diseases, and tolerance to abiotic stress (Gomes et al., 2015; Zeilinger et al., 2016). On the other hand, other species of *Trichoderma*, such as *Trichoderma reesei* and *Trichoderma longibrachiatum* are recognized as industrial enzyme producer and human immunocompromised opportunistic fungus (Kubicek et al., 2011).

In this review, we will discuss the interactions that the *Trichoderma* genus has with plants, as well as mechanisms of the biological control of plant pathogens in pre-harvest crops. In addition, we will highlight how these interactions can influence crop production and food security.

2. Plant fungal pathogens in pre-harvest crops

Fungi are predominant among plant pathogens as agents in plant diseases, and can cause enormous losses in crop yield and quality. This is becoming an important issue for both human health and food security. Fungal plant pathogen species include members from the phyla Ascomycota, as well as Basidiomycota (Doehlemann et al., 2017).

Fungal phytopathogens have developed different modes of interaction with their host plants. Those that synthesize and secrete toxic secondary metabolites as the first resources for colonization, killing host cells and thriving on organic compounds, are named necrotrophic. Conversely, fungi that live off nutrients provided by

living hosts for prolonged periods of time and do not produce toxins are called biotrophic (Zeilinger et al., 2016). Pathogens exhibiting a combination of these two lifestyles and nutritional strategies, wherein pathogens exhibit a transient biotrophic life period followed by a necrotrophic lifestyle, are called hemi-biotrophic (Zeilinger et al., 2016).

Fungal infections can cause a variety of diseases in different crops. These include *Botrytis cinerea* (grey mould on fruits like grapes and strawberries), *Pythium ultimum* (seed rots and damping-off, root, stem and fruit rots, foliar blights, and post-harvest decay of various host plants, including corn, soybeans, potatoes, and wheat), *Fusarium oxysporum* (vascular wilt of the banana tree), *Sclerotinia sclerotiorum* (soft rot in bean and soybean crops), *Ustilago maydis* (maize smut in maize crops), *Cladosporium fulvum* (tomato leaf mould), *Phytophthora infestans* (potato late blight), *Rhizoctonia solani* (damping-off in beans, soybeans, cotton, and rice crops), and *Macrophomina phaseolina* (damping off, seedling blight, collar rot, stem rot, charcoal rot, basal stem rot, and root rot in peanuts, cabbage, pepper, chickpeas, soybeans, sunflowers, sweet potatoes, alfalfa, sesame, potatoes, sorghum, wheat, and corn) (Akino et al., 2004; Babu et al., 2007; Bolton et al., 2006; Cheung et al., 2008; Choquer et al., 2007; Gordon and Martyn, 1997; Rivas and Thomas, 2005; Snetselaar and Mims, 1992).

The top ten fungal pathogens in molecular plant pathology were reviewed by Dean et al. (2012) and Doehlemann et al. (2017), based on scientific/economic importance. The list includes (1) *Magnaporthe oryzae*; (2) *B. cinerea*; (3) *Puccinia* spp.; (4) *Fusarium graminearum*; (5) *F. oxysporum*; (6) *Blumeria graminis*; (7) *Mycosphaerella graminicola*; (8) *Colletotrichum* spp.; (9) *U. maydis*; and (10) *Melampsora lini*. Table 1 summarizes the fungal diseases and crops affected, as well some symptoms.

3. Biological control strategies by *Trichoderma*

The genus *Trichoderma* comprises the imperfect phase of *Hypocrea*, belonging to the Kingdom Fungi, Phylum Ascomycota, Class Ascomycetes, Order Hypocreales, Family Hypocreaceae. The genus was proposed by Persoon in 1794 for those fungi that possessed the following set of well-defined characteristics: rapid growth in culture medium; dispersed, floccose, or tufted compacts; size and shape of the various conidia; chlamydospores, sometimes present; and coloring of conidia varying from green to yellow, or even hyaline, with well-defined conidiophores and conidia formed at the phyalid ends of differentiated hyphae, tending towards mass aggregation (Samuels, 1996). It comprises a group of fungi present in almost all soil types, especially those containing organic matter

Table 1
The top 10 Fungal disease infecting crops in pre-harvest.

Fungal disease	Causing agent	Crops affected	Spreading-factors	Symptoms	Reference
Rice blast	<i>Magnaporthe oryzae</i>	rice and wheat	High relative humidity Temp. 25–27.7 °C	white to gray-green lesions or spots with darker borders produced on all parts of the shoot	Couch et al. (2005)
Grey mould	<i>Botrytis cinerea</i>	Celery; lettuce; beans; capsicum; tomato	Cool, wet weather	soft rot, soft fruit and leaves. Brown lesion	Williamson et al. (2007)
Rusts	<i>Puccinia</i> spp	Sweet corn; beans; onions; spring onions; beets;	low rainfall, 100 % relative humidity and cool to mild temperatures	Small, red or reddish- brown pustules	Van Baarlen et al. (2007)
Fusarium head blight	<i>Fusarium graminearum</i>	wheat, barley, oats, rye and triticale	Warm, humid weather;	shriveling kernels	Wegulo et al. (2015)
Fusarium wilt	<i>Fusarium oxysporum</i>	Tomato, tobacco, legumes, cucurbits, sweet potatoes and banana	Warm to hot weather	vascular browning, leaf epinasty, stunting, progressive wilting, defoliation and plant death	Fravel et al. (2003)
Powdery mildew	<i>Blumeria graminis</i>	wheat and barley	low humidity and moderate temperatures	white powdery spots on the leaves and stems	Nowara et al. (2010)
Septoria Tritici Blotch (STB)	<i>Mycosphaerella graminicola</i>	wheat	temperate regions	necrotic blotches on the foliage	Orton et al. (2011)
Anthracnose	<i>Colletotrichum</i> spp	bananas, cassava, sorghum, coffee, strawberry, common bean	Cool, wet conditions	anthracnose spots and blights of aerial plant parts	Prusky (1996)
Corn smut	<i>Ustilago maydis</i>	maize and teosinte	plant environment condition	causes the corn kernels to swell up into tumor-like galls	Holliday (2004)
Flax rust	<i>Melampsora lini</i>	F lax, linseed, wheat	Temperate plains or hills	Yellowing of leaves Necrotic leaf spots	Lawrence et al. (2010)

(Harman et al., 2004a). Some species of fungi of the genus *Trichoderma* are dominant components in the microflora present in a wide variety of habitats. This is a special feature, due to its great metabolic capacity and its aggressively competitive nature (Kubicek et al., 2008; Lopes et al., 2012).

Widely used as biocontrol agents in agriculture, *Trichoderma* spp. can induce a combination of antagonistic mechanisms, such as: antibiosis through the production of secondary metabolites with anti-fungal activity; mycoparasitism, with the production of cell wall-degrading enzymes from plant pathogens, due to competition for nutrients or space; and induction of resistance in plants through the production and secretion of elicitor molecules (Gomes et al., 2015). The general mechanisms of the biocontrol of the *Trichoderma* spp. can be divided into direct and indirect effects. Direct effects include competition for nutrients or space, production of volatile and non-volatile antibiotics and lytic enzymes, inactivation of pathogen enzymes, and parasitism. Indirect effects include morphological and biochemical changes in the host plants, such as stress tolerance, solubilization or sequestration of inorganic nutrients, and induction of resistance to diseases caused by fungal phytopathogens (Viterbo et al., 2002) (Fig. 1).

Some *Trichoderma* spp. are efficient in colonizing the surface of plant roots, leading to large changes in plant metabolism. This effect has been reported in some *Trichoderma* spp., which favors plant growth, increases nutrient availability, and increases disease resistance (Harman et al., 2004a). Elicitor molecules produced by *Trichoderma* activate the expression of genes involved in the plant defense system, and promote plant growth, roots, and nutrient availability (Gomes et al., 2017). In greenhouses, *Trichoderma* spp., especially *Trichoderma harzianum* T22 and *Trichoderma atroviride* P1, have been well studied for being good promoters for the growth of lettuce, tomato, and pepper. They have been shown to increase the productivity in 300 % of the treated groups compared to the untreated ones (Vinale et al., 2004).

Resistance induction is an indirect biological control mechanism, wherein the plant responds to the aggression of the pathogens through activation of latent resistance mechanisms. This

process occurs when plants exposed to an inductive agent, biotic or abiotic, activate their defense mechanisms in a relatively generalized way, not only in the induction site but also in other distant locations. This activation can last for variable periods of time, and the plant may produce phytoalexins, additional lignin from cells, and phenolic compounds (Bailey et al., 2009; Rocha et al., 2017).

The term “secondary metabolites” refers to a group of different natural chemical compounds possibly related to survival functions, such as competition against microorganisms, symbiosis, metal transport, differentiation, and antibiosis (Vinale et al., 2008a). The first study on the toxic metabolites produced by *Trichoderma* spp. was by Weindling (1934), who reported the control of plant diseases by a “lethal principle” produced by *Thielaviopsis lignorum*. This was later known as the antibiotic gliotoxin. Weindling described *T.lignorum* mycoparasitism in detail against *R. solani*, revealing the potential of *Trichoderma* spp. as biocontrol agents in plant diseases (Howell, 2003a). Preliminary work to understand the role of antibiotics produced by *Trichoderma* spp. in plant pathogen biocontrol was carried out by Dennis and Webster (1971). In this study, trichodermine and antibiotic peptides from culture extracts of *Trichoderma* spp. secreted a diverse range of secondary metabolites, and their chemical characteristics and antimicrobial properties have been studied. Howell, Stipanovic, and Lumsden (1993) isolated and described *Gliocladium gliovirine* (*Trichoderma virens*) as a potent inhibitor of *P. ultimum* and *Phytophthora*, but determined it did not exert any inhibitory activity against *R. solani*, *Thielaviopsis basicola*, and *Phymatotrichum*, among others. It also did not have any activity against some bacteria, such as *Bacillus thuringiensis*. Schirbock et al. (1994) investigated the performance of Trichorzianins of *T. harzianum* as an antibiotic model against *B. cinerea*. The authors showed that both enzyme and antibiotic synthesis were directed to the cell wall of *B. cinerea*, and that the antibiotic acts in synergism with chitinases and glucanases, inhibiting sporulation, germination, and stretching of the fungal hyphae. It has also been described that alkyl pyrones are responsible for the strong coconut odor in *Trichoderma viride* species, where the 6-pentyl pyrone compound is active against a variety of phytopathogens

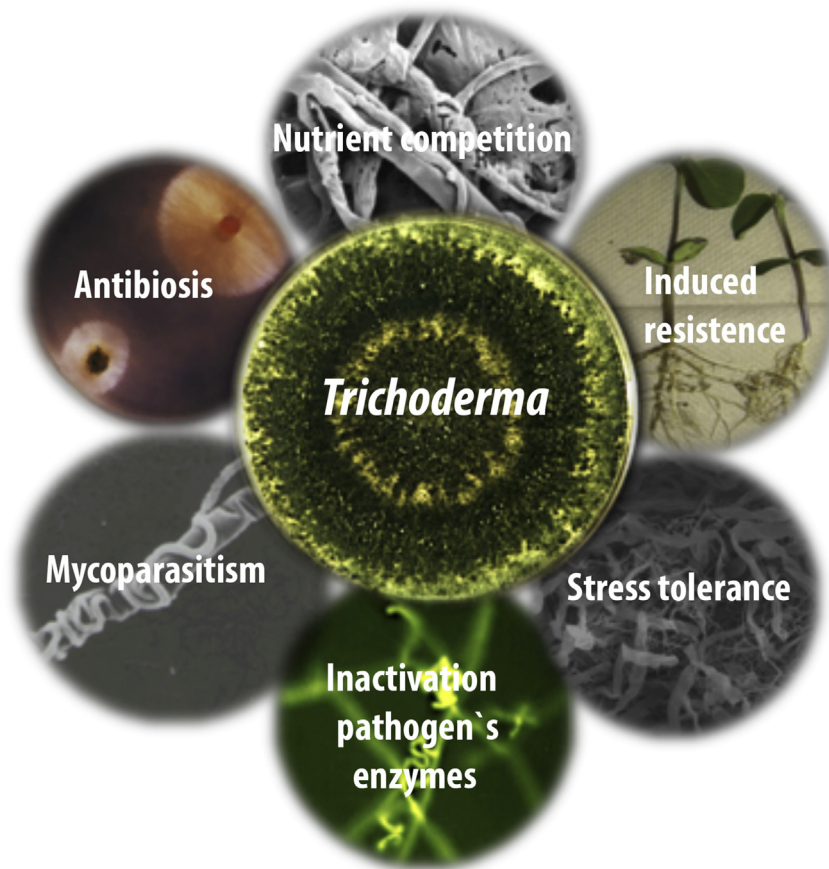


Fig. 1. Strategies used by the genus *Trichoderma* during biocontrol.

(Schirmbock et al., 1994). It has also been isolated from other species, such as *T. harzianum*, *Trichoderma koningii*, and *Trichoderma hamatum* (Vinale et al., 2008a). Another class of antibiotics is the isonitriles, produced by *Trichoderma* spp. These include isonitrine A-D and isonitric acid E and F, isolated from *T. hamatum*, *T. harzianum*, *T. koningii*, *Thielaviopsis polysporum*, and *T. viride* (Adelin et al., 2017). Isonitrin A is effective against Gram positive and Gram negative bacteria, while Isonitrin D shows good fungal activity and no activity against bacteria (Howell, 2003b). Studies with the application of a racemic form of harzianopyridone demonstrated its potent antifungal activity against *R. solani* and *B. cinerea* (Cutler and Jacyno, 1991). Secondary metabolites such as T22-azaphilone, harzianolide, and T39 butenolide from specific *Trichoderma* strains have shown *in vitro* inhibition of *R. solani*, *P. ultimum*, and *G. graminis* var. *tritici* (Almassi et al., 1991; Vinale et al., 2006).

The competition, on the other hand, refers to the interaction between two or more organisms engaged in the same action or substrate, disputing specific resources such as space, nutrients, water, and light (Benítez et al., 2004). As illustrative examples of this mechanism, *Trichoderma* spp. are able to readily mobilize and absorb the nutrients around them and use different carbon sources, thereby rapidly multiplying and colonizing the rhizosphere (Harman et al., 2004a). Moreover, several species of this genus are characterized by resistance to different toxic compounds, both those produced and released by plants in response to attack by pathogens, and agrochemicals commonly used in agriculture (Chet and Inbar, 1994; Harman, 2006).

Mycoparasitism is undoubtedly the most characteristic behavior of the *Trichoderma* spp. Mycoparasitism is the ability to parasitize

other fungi, and is a complex process involving four distinct stages: (a) chemotropic growth, in which a chemical stimulus attracts the antagonistic fungus; (b) specific recognition, probably mediated by lectins on the cell surface of both the pathogen and antagonist; (c) attack and coiling of *Trichoderma* around the host hyphae; and (d) secretion of lytic enzymes that degrade the host cell wall (Vinale et al., 2008a). During the process of mycoparasitism, *Trichoderma* secretes cell wall-degrading enzymes (CWDEs) that will degrade the cell wall of the host fungus. This will then release oligomers, activating the expression of genes involved in mycoparasitism (Almeida et al., 2007). Evidence for this recognition comes from studies on transcriptomics, which show the induction of CWDE genes before actual contact with *B. cinerea* (Mukherjee et al., 2012b; Seidl et al., 2005).

Some enzymes involved in mycoparasitism are released in response to the cell wall of most phytopathogens fungi, which have chitin and or glucan fibrils embedded in a protein matrix (Bartnicki-Garcia, 1968). Thus, lysing of the cell wall of phytopathogens is mainly done by glucanases, chitinases, and proteases (Monteiro et al., 2010; Naher et al., 2014). Other CWDEs that degrade smaller polymers, such as β -1,6-glucanases, β -1,3-glucanases, and mannosidases, may be involved in the complete and effective degradation of the cell wall of plant pathogens by *Trichoderma* spp. (Monteiro et al., 2010; Saba, 2012). β -1,3-glucanases are enzymes that catalyze the hydrolysis of the β -1,3-glucan chain, a polymer composed of β -glucose residues bound in a β -1,3 configuration. They are cleaved into the following compounds: exo- β -1,3-glucanases (EC 3.2.1.58), which sequentially hydrolyze β -1,3 glycosidic bonds at the non-reducing end of the glucan molecule, releasing glucose as the end product; and endo- β -

1,3- glucanases (EC 3.2.1.39) that randomly cleave β -1,3 bonds along the polysaccharide chain by releasing small oligosaccharides, with glucose as the final product (Monteiro and Ulhoa, 2006). It is possible that synergistic action occurs between at least two enzymes, with different modes of action in fungi, that degrade β -glucans (Ait-Lahsen et al., 2001). According to the CAZY databases, the exo- β -glucanases (EC 3.2.1.58) were distributed in the GH families 3, 5, 17, and 55, while the endo- β -glucanases (EC 3.2.1.39) are in the GH families 16, 17, 55, 64, and 81 (Druzhinina et al., 2011).

Another important enzyme class in mycoparasitism is chitinase. The best characterized chitinolytic system of *Trichoderma* species is from *T. harzianum* and *T. atroviride* presenting a complex system of more than six chitinolytic enzymes, endochitinases and two N-acetylhexosaminidases (Ulhoa and Peberdy, 1991). Chitinases include endo- and exochitinases where the endochitinases cleave the chitin molecule internally in chitotetraose, chitotriosis and diacetylchitobiose, and the exochitinases, that are subdivided in chitobiosidases and N-acetyl-D-glucosaminidases. Chitobiosidases catalyze the progressive liberation of diacetylchitobiose and N-acetyl-D-glucosaminidases hydrolyze diacetylchitobiose in monomers of N-acetylglucosamine (Gruber et al., 2011). According to the CAZY databases, chitinases are glycosyl hydrolases allocated in the GH 18, GH 19, and GH 20 families (Hjort et al., 2010). Antifungal activity and mycoparasitism studies are well described for some plant pathogens, such as *B. cinerea*, *R. solani*, *Fusarium solani*, and *S. sclerotiorum* (Almeida et al., 2007; Lopes et al., 2012).

Proteases can also participate in the degradation of structural cellular proteins, destabilizing the cellular integrity of the phytopathogen and facilitating penetration and colonization by *Trichoderma* (De Marco and Felix, 2002). They are also involved in the inactivation of enzymes produced by pathogens during the plant infection process (Suárez et al., 2007). Despite its importance for mycoparasitism, the number of protease characterization, isolation, and/or cloning studies is lower than studies related to chitinases and β -1,3-glycanases. However, the genes of some serine endopeptidases (p8048, ss10) (Suárez et al., 2007; Liu et al., 2009) and aspartic proteases (*papA*, p6281) (Delgado-Jarana et al., 2002; Suárez et al., 2005) seem to be involved in the control of some plant pathogens. Others proteins involved in mycoparasitism have been described using 'omics' approaches (Adav and Sze, 2014; Monteiro et al., 2010; Ramada et al., 2016; Tian et al., 2009). In addition to glucanases, chitinases and proteases, other enzymes such as α -galactosidase, α -1,2-mannosidase, α -L-arabinofuranosidase, mutanase and β -glucocerebrosidase have been identified by proteomic approach. Table 2 summarizes the most important enzymes involved in the mycoparasitism process.

4. Molecular tools for *Trichoderma*/pathogen studies: 'omics' studies

The first fungal genomics milestone was the publication of the whole genome sequence of the yeast *Saccharomyces cerevisiae* (Goffeau et al., 1996). This organism has played an exceptional role in expanding our basic knowledge of eukaryotic cell physiology, with its ~6000 genes. The first *Trichoderma* strain that had its genome sequenced was the *T. reesei* (Martinez et al., 2008), the industrial workhorse regarding cellulase production. Other species of *Trichoderma* garnered attention due to the excellent ability of its species to suppress diseases and stimulate the growth and development of plants (Pereira et al., 2014).

The advent of high-throughput technologies, especially regarding to next generation techniques (NGS), has led to a wealth of publicly available 'omics' data coming from different experimental sources, such as transcriptomics, proteomics, and metabolomics. Single strategies or combining different biological

datasets (dos Santos Castro et al., 2014) can lead to the discovery of important biological insights, especially in complex microorganism interactions. The addition of 'omics' to a molecular term implies a comprehensive or global assessment of a set of molecules (Hasin et al., 2017). The first 'omics' discipline to appear, genomics, focused on the study of whole genomes, as opposed to "genetics" that focus on individual variants or single genes. The 'omics' field has been mainly driven by technological advances that have made the cost-efficient, high-throughput analysis of biological molecules possible (Hasin et al., 2017). Fig. 2 shows the 'omics' combined strategies that can be used to study *Trichoderma*/pathogen interaction.

Combining large-scale initiatives of *Trichoderma* genome sequencing (Druzhinina et al., 2018) with single genomes from labs around the world (Table 3) is found in the National Center for Biotechnology Information (NCBI) Genome Project databank (www.ncbi.nlm.nih.gov/genome/). Sixteen genomes from different species of *Trichoderma* are publicly available on the NCBI/genbank database (Table 3). A good representation of the three major sections of this genus, *Pachybasium*, *Longibrachiatum*, and *Trichoderma*, are available as demonstrated in the single-copy ortholog phylogenetic tree (Fig. 3).

With the advent of Sangerexpressed sequence tag (EST) projects around a decade ago, it became possible to study a higher number of transcripts from *Trichoderma* during its interaction with phytopathogens (Seidl et al., 2009a,b; Steindorff et al., 2014; Vizcaíno et al., 2007). Despite this approach being sold as "high-throughput", it usually generates around 1000 unique sequences per library, which represents ~10 % of total *Trichoderma* genes (considering average total gene count of 10,000 in *Trichoderma*). Other techniques such as suppression subtractive hybridization (SSH) were used to detect genes present only in *Trichoderma* in the presence of phytopathogen cell walls (Vieira et al., 2013).

All these studies (Seidl et al., 2009a,b; Steindorff et al., 2012; Vieira et al., 2013; Vizcaíno et al., 2007) found a similar pattern of genes involved in the response of *Trichoderma* to the presence of phytopathogens, representing post translational processing and amino acid metabolism. These included components of the stress response, reaction to nitrogen shortage, signal transduction, lipid catabolism pathogenicity factors, proteases, and a QID74/CFEM protein considered to be involved in cell wall protection and appressorium development.

Microarrays for expression profiling were used to study *Trichoderma*/pathogen interaction (Atanasova et al., 2013). They compared the transcriptional responses of *T. atroviride*, *T. virens*, and *T. reesei* during confrontations with a plant pathogenic fungus, *R. solani*. The three *Trichoderma* spp. exhibited different transcriptomic responses already before physical contact with phytopathogens. *T. atroviride* expressed genes involved in the production of secondary metabolites, β -glucanases, various proteases, and small secreted cysteine-rich proteins (SSCP). *T. virens*, on the other hand, mainly expressed genes involved in the biosynthesis of gliotoxin and glutathione. In contrast, *T. reesei* increased the expression of genes encoding cellulases and hemicellulases, and of genes involved in solute transport (Atanasova et al., 2013). The development of next-generation sequencing (NGS) methods again rapidly changed the possibilities for studying gene expression, through mapping to a reference genome and developing whole genome expression profiles, in addition to introducing the possibility of using NGS directly to sequence and assemble transcriptomes (Kohler and Tisserant, 2014). Steindorff and collaborators used Illumina sequencing to analyze the interaction between *T. harzianum* and the phytopathogen *F. solani*. They identified various genes of biotechnological value, encoding proteins with functions such as proteases, transporters, glycosyl

Table 2The most important enzymes involved in the mycoparasitism process by *Trichoderma*.

Source	Enzyme	Molecular Weight (kDa)	Reference	
Glucanases obtained by chromatography	Exo-1,3- β	75	Dubourdieu et al. (1985)	
	Exo-1,3- β	31	Kitamoto et al. (1987)	
	Endo -1,3- β	76	Lorito et al. (1994)	
	Endo -1,3- β	36	De La Cruz et al. (1995)	
	Exo-1,3- β	110	Cohen-Kupiec et al. (1999)	
	Endo-1,3- β	40	Noronha et al. (2000)	
	Exo-1,3- β	29	Noronha et al. (2000)	
	Exo- β -1,3-	83.1	Bara et al. (2003)	
	Endo- β -1,6-	46	Monteiro and Ulhoa (2006)	
	Exo-1,3- β	78	Monteiro and Ulhoa (2006)	
	Exoglucanase (EXG Th1).	61	Liu et al. (2013)	
	Endoglucanase (EG Th1)	23.5	Liu et al. (2013)	
	α -(1 \rightarrow 3)-glucanase	67	Wiater et al. (2013)	
	Chitinases obtained by chromatography	N-acetylglucosaminidase	102–118	Ulhoa and Peberdy (1991)
		Endoquitinase	33–37	DeLa Cruz et al. (1992)
				Ulhoa and Peberdy (1991)
		Exoquitinase	40	Harman (1993)
N-acetylglucosaminidase		73	Harman (1993)	
			Lorito et al. (1994)	
Endoquitinase		52	Harman (1993)	
Endoquitinase		31–33	DeLa Cruz et al. (1992)	
Endoquitinase		46	Lima et al. (1997)	
Other enzymes found in secretoma		α -mannosidase	53.52	Monteiro et al. (2010)
	Acid phosphatase	41.71	Monteiro et al. (2010)	
	α -1,3-Glucanase	71.79	Monteiro et al. (2010)	
	Carboxypeptidase 2	53.79	Monteiro et al. (2010)	
	Glucosidase 1	27.50	Monteiro et al. (2010)	
	α -mannosidase	53.52	Monteiro et al. (2010)	
	Carboxypeptidase 2	53.45	Monteiro et al. (2010)	
	Endochitinase	41.71	Monteiro et al. (2010)	
	α -l-arabinofuranosidase	ND	Ramada et al. (2016)	
	Endo-1,3(4) - β glucanase	ND	Ramada et al. (2016)	
	Endochitinase chit33	33	Ramada et al. (2016)	
	chit37 Endochitinase	37	Ramada et al. (2016)	
	chit42 Endochitinase	42	Ramada et al. (2016)	
	α -Galactosidase	ND	Ramada et al. (2016)	
	α -1,2-mannosidase	ND	Ramada et al. (2016)	
	β -1,6-glucanase	ND	Ramada et al. (2016)	
	α -1,3-glucanase	ND	Ramada et al. (2016)	
	β -endo-1,3-glucanase	ND	Ramada et al. (2016)	
	Endo- β -1,4-glucanase	ND	Ramada et al. (2016)	
	Trypsin-like protease	ND	Ramada et al. (2016)	
	Serine protease	ND	Ramada et al. (2016)	
	aspartate protease	ND	Ramada et al. (2016)	
	Mutanase	67.63	Blauth de Lima et al. (2017)	
	Beta 1,3 exoglucanase	107.93	Blauth de Lima et al. (2017)	
	endochitinase	42	Blauth de Lima et al. (2017)	
	Serine endopeptidase	42.47	Blauth de Lima et al. (2017)	
	Glucoamylase	66.25	Blauth de Lima et al. (2017)	
	Endochitinase	34.026	Blauth de Lima et al. (2017)	
	β -1,3 exoglucanase	107.28	Kohler and Tisserant (2014)	
	endo-1,3- β glucanase	92.19	Nauom et al. (2018)	
	Six-hairpin glycosidase-like	76.55	Nauom et al. (2018)	
	1, 2- α -mannosidase	55.65	Nauom et al. (2018)	
	Peptidase S8	92.55	Nauom et al. (2018)	
	α -D-galactosidase	48.25	Nauom et al. (2018)	
	1,4- α -glucosidase	67.28	Nauom et al. (2018)	
	Tyrosinase	46.95	Nauom et al. (2018)	
	protein β -1,3 glucanase	40.1	Nauom et al. (2018)	
peptidase M14	46.95	Nauom et al. (2018)		
β -glucocerebrosidase	51.59	Nauom et al. (2018)		

hydrolases, adherence, appressorium development, and pathogenesis (Steindorff et al., 2014).

On the other hand, the analysis of whole proteomes has only been possible with the advent of mass spectrometry-based methods. The proteome *Trichoderma*/pathogen interactions started with classical two-dimensional electrophoresis, where “spots” from each acrylamide gel were excised and digested with trypsin in order to sequence tryptic peptides through spectrometric analysis, using

matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) analysis. Studies then went to a more sophisticated liquid chromatography-tandem mass (LC-MS-MS) to separate peptides (Marra et al., 2006; Monteiro et al., 2010; Nauom et al., 2018; Pereira et al., 2014; Ramada et al., 2016). Marra et al. (2006) used two-dimensional (2-D) electrophoresis to separately analyze collected proteomes from each single, two-, or three-partner interaction (i.e., plant, pathogenic, and antagonistic fungus alone, and in all possible

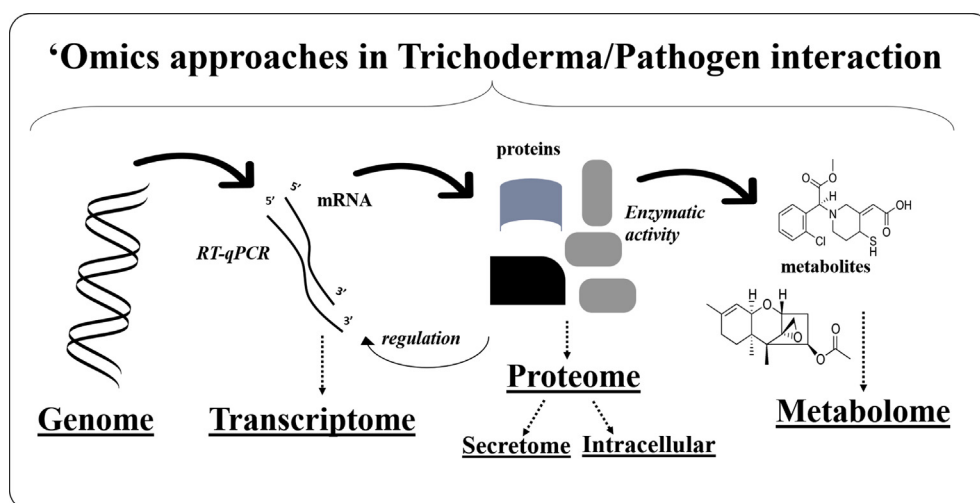


Fig. 2. 'Omics' strategies to study *Trichoderma*/pathogen interaction.

Table 3

Genome features of *Trichoderma* genomes publicly available at NCBI/Genbank.

Species	Strain	Genome size	Gene count	Reference
<i>T. reesei</i>	QM6a	32.7	9877	Martinez et al. (2008)
<i>T. longibrachiatum</i>	ATCC18648	31.74	10 938	Druzhinina et al. (2018)
<i>T. citrinoviride</i>	TUCIM 6016	33.2	9737	Druzhinina et al. (2018)
<i>T. parareesei</i>	CBS125925	32.07	9292	Yang et al. (2015)
<i>T. harzianum</i>	CBS 226.95	40.9	14 095	Druzhinina et al. (2018)
	TR274	39.4	13 932	
<i>T. arundinaceum</i>	IBT40837	36.87	10 473	Proctor et al. (2018)
<i>T. atroviride</i>	ITEM 908	39.15	8649	Fanelli et al. (2018)
<i>T. koningiopsis</i>	POS7	36.58	12 661	Castrillo et al. (2017)
<i>T. koningii</i>	JCM 1883	32.32	–	
<i>T. pleuroti</i>	TPhu1	38.14	–	
<i>T. guizhouense</i>	NJAU4742	38.8	11 297	Druzhinina et al. (2018)
<i>T. virens</i>	Gv29-8	40.52	12 427	Kubicek et al. (2011)
<i>T. atroviride</i>	IMI 206040	36.4	11 863	Kubicek et al. (2011)
<i>T. gamsii</i>	T6085	37.9	10 709	Baroncelli et al. (2015)
<i>T. asperellum</i>	CBS433.97	37.66	12 586	Druzhinina et al. (2018)
<i>T. hamatum</i>	GD12	38.43	10 520	Studholme et al. (2013)

combinations). In the plant proteome, specific pathogenesis-related proteins and other disease-related factors (i.e., potential resistance genes) seem to be associated with the interaction with either *T. atroviride* and/or pathogens. On the other hand, in the *T. atroviride* interaction proteome, a fungal hydrophobin and ABC transporters were found. Pereira et al. (2014) evaluated the ability of *T. harzianum* to promote common bean growth and to modulate its metabolism and defense response, in the presence or absence of the phytopathogenic fungi *R. solani* and *F. solani*, using a proteomic approach. *T. harzianum* was able to promote common bean plant growth, as shown by the increase in root/foiar areas and by its size in comparison to plants grown in its absence. The interaction appeared to modulate the expression of defense-related genes (*glu1*, *pod3*, and *lox1*) in roots of *Phaseolus vulgaris*.

Identification of *T. harzianum*-secreted proteins (secretome) grown on phytopathogen cell walls (mycoparasitism simulation) through MS-based analysis was used to understand the interaction. Monteiro et al. (2010) identified seven proteins using MASCOT search with associated functions, such as α -1,3-glucanase, carboxypeptidase 2, glucosidase I, α -mannosidase, acid-phosphatase, and an endochitinase (Table 2). Ramada et al. (2016) (Ramada et al., 2016) used a similar approach using *T. harzianum* grown on *F. solani* cell walls. In this study, a manual sequencing of MS-MS spectra was used. This laborious method yielded 97 spots (from a total of 105) using MS

spectra, with good ion intensity. 94 proteins from 37 different genes were identified in this study, including 22 CAZymes, 11 proteases, and 4 proteins with other functions, such as NPP1 and Epl-1. The latter was studied in more detail, and it was revealed that this protein is involved in mycoparasitism, plant resistance induction, and self-cell wall protection (Gomes et al., 2017, 2015).

In order to survive and compete in their ecological niche, fungi apply not only enzymatic weapons but also have a potent arsenal for chemical warfare at their disposal (Vinale et al., 2008b). Thereby, not only potential antibiotics (e.g. peptaibols) but also mycotoxins and more than 100 metabolites with antibiotic activity were detected in *Trichoderma* spp., including polyketides, pyrones, terpenes, metabolites derived from amino acids, and polypeptides (Brito et al., 2014). It was described that secondary metabolites (SM) result in specific communication between the microorganisms (Netzker et al., 2015). SM plays a key role in this communication, and it was shown that interspecies "talk" between microorganisms represents a physiological trigger to activate silent gene clusters, leading to the formation of novel SMs by the involved species (Netzker et al., 2015). Therefore, a larger repertoire of SM could represent a more diverse "vocabulary" during the interaction between different microorganisms.

Fungi produce a wide range of SMs, and *Trichoderma* is a good source of such molecules. The production of these compounds by

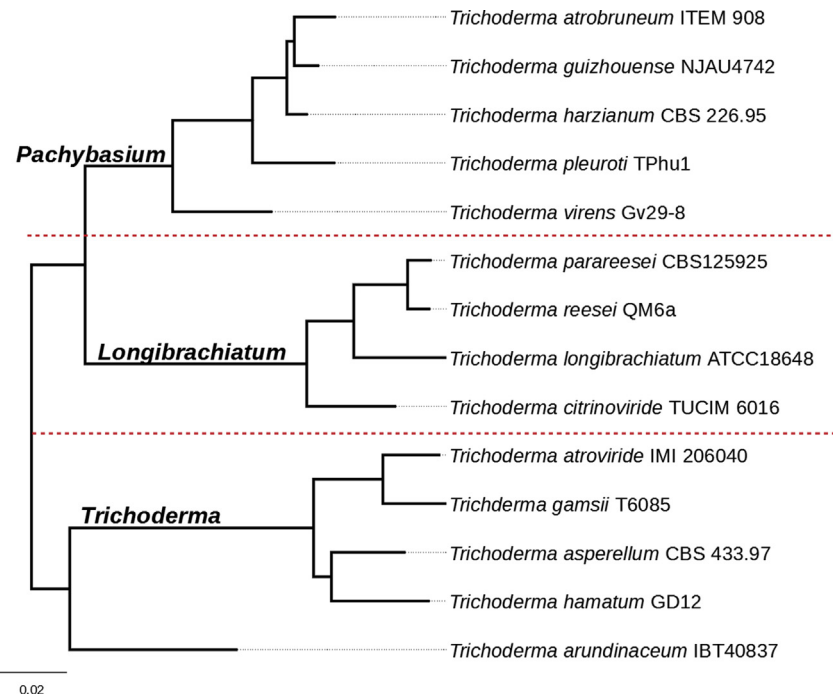


Fig. 3. Maximum likelihood phylogenetic tree using 5030 single-copy orthologs present in all *Trichoderma* strains. The red dotted line separates the three major sections in *Trichoderma* genera: *Pachybasium*, *Longibrachiatum*, and *Trichoderma*. All nodes have maximum support value. Scale bar represents amino acids substitutions per site. The tree was rooted using the midpoint. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Trichoderma spp. is strain-dependent, and includes different classes of antifungal compounds, like volatile antibiotics, water-soluble compounds, peptaibiotics, and peptaibols. Britto et al. (Britto et al., 2014) identified seven different peptaibols (asperelines and trichotoxins) in *Trichoderma asperellum*, grown in a simple medium and using only glucose as a carbon source. An example of the application of these secondary metabolites is the application of harzianic acid (HA) in tomato plants, stimulating the response of tomatoes to the pathogen. This is done by inducing the expression of several genes involved in the defense response (including protease inhibitors, resistance proteins like CC-NBS-LRR) and hormone interplay (Pascale et al., 2017). Table 4 shows the diversity of secondary metabolite clusters on the six organisms with its genomes recently published (Druzhinina et al., 2018).

Interestingly, the genomes of mycoparasitic species are enriched in virtually all types of SMs. The majority of computationally identified fungal SM gene clusters are silent under standard laboratory growth conditions (Mukherjee et al., 2012b). The availability of new genomes reveals an excellent opportunity to study and compare SM clusters in a vast array of species, and potentially discover new functional compounds.

5. Interaction mechanism of *Trichoderma*/pathogens/plants

Trichoderma spp. are soil-borne fungi characterized by their saprophytic, mycoparasitic, and symbiotic lifestyles. Symbiotic *Trichoderma* spp. interact directly with host plants, being able to colonize their roots and promoting plant growth, tolerance to abiotic stress, or resistance to further infections (Brotman et al., 2012; Contreras-Cornejo et al., 2011; Mukherjee et al., 2012b; Shores et al., 2010). In addition, these species protect against pathogens in an indirect way as a result of their direct action against plant pathogens. Presently, the main goal is to provide an overview of the mechanism and molecular players involved in interactions between host plants and *Trichoderma* spp., especially *T. harzianum*, *T. atroviride*, *T. virens*, and *T. asperellum*.

A set of proteins and metabolites has been mapped and predicted based on the secretomes of *Trichoderma* spp., during interaction with host plants (Nogueira-Lopez et al., 2018; Lamdan et al., 2015; Morán-Diez et al., 2015; Hermosa et al., 2013; Mendoza-Mendoza et al., 2018; Druzhinina et al., 2012). The three groups mainly represented are carbohydrate active enzymes, including plant cell wall and fungal cell wall-degrading enzymes, proteases,

Table 4

The number of secondary metabolites clusters found on recently published *Trichoderma* genomes.

	Hybrid PKS/NRPS	NRPS	PKS	Terpene cyclases
<i>Trichoderma asperellum</i> CBS 433.97	2	24	14	4
<i>Trichoderma atroviride</i> IMI 206040	2	19	15	3
<i>Trichoderma harzianum</i> CBS 226.95	5	23	23	6
<i>Trichoderma virens</i> Gv29-8	2	31	19	6
<i>Trichoderma longibrachiatum</i> ATCC 18648	1	12	12	3
<i>Trichoderma reesei</i> QM6a	2	13	11	4

Adapted from (Druzhinina et al., 2018).

and small cysteine-rich secreted proteins. These proteins might play a role in the mechanisms of interaction between symbiotic *Trichoderma* spp. and their hosts in colonization, plant growth promotion, or modulation of the defense response. However, their effective participation in these processes is still uncertain, and must be further elucidated.

Appressoria-like structures favor attachment of the fungus to the host roots, enabling tissue penetration by the hyphae of *Trichoderma* spp., usually limited to the intercellular spaces of roots and restricted to the epidermis (Hermosa et al., 2012; Yedidia et al., 1999). Swollenins, hydrophobins, and SM2 are classified as part of the small secreted cysteine-rich proteins family, and have been demonstrated to be critical to colonization of host roots by *Trichoderma* spp. *T. asperellum* mutants for a class I hydrophobin (TASHYD) severely impaired the ability of cucumber (*Cucumis sativus*) roots to attach and colonize, and this ability was restored in complemented mutants (Viterbo and Chet, 2006). Hydrophobin also participates in the colonization of tomato plant roots by *T. virens*. Resistance induction is an indirect biological control mechanism, wherein the plant responds to the aggression of the pathogens through the activation of latent resistance mechanisms. This process occurs when plants are exposed to an inductive agent, biotic or abiotic, and their defense mechanisms are activated in a generalized manner, both in the induction site and other distant locations. This can last for variable periods of time, and the plant may produce phytoalexins, additional lignin from cells, and phenolic compounds (Bailey et al., 2009; Rocha et al., 2017).

The authors showed that overexpression of a gene that encoded a hydrophobin class II, tvhdyii, leads to an increased ability to colonize host roots, while its deletion decreases it (Guzmán-Guzmán et al., 2017).

The role of a swollenin in root colonization was showed by observing the interaction between *T. asperellum* and cucumber. Fungal transformants over-expressing the swollenin-encoding gene, *tasswo*, displayed a remarkably increased ability to colonize cucumber roots 6 h after inoculation (Brotman et al., 2008). The protein contains a carbohydrate-binding domain (CBD) able to recognize and interact with cellulose in plant cell walls connected by a linker region to an expansin-like domain. Expansins have been described in other fungi as acting on an extension of plant cell walls, by weakening the non-covalent interactions that help to maintain its integrity. Therefore, TASSWO may modify plant cell wall architecture, favoring root colonization by *T. asperellum*. The protein SM2 is highly expressed by *T. virens*, grown in association with maize. Deletion of its encoding gene leads to a lowered ability to colonize maize roots (Crutcher et al., 2015).

Plant CWDEs secreted during host plant and *Trichoderma* spp. interaction, in turn, allow root penetration by thickening the plant cell wall (Hermosa et al., 2012). This role was described for an endopolygalacturonase, which was differentially upregulated during interaction of *T. harzianum* with tomato plants. Silencing of the enzyme-encoding gene, *thpg1*, resulted in a significant decrease of fungal root colonization activity (Morán-Diez et al., 2009). Using *Arabidopsis* as a model organism, Martínez-Medina et al. (2017) showed that in addition to the previously mentioned proteins, the level of salicylic and jasmonic acid also influences root colonization by *T. harzianum* T-78. An increased level of salicylic acid prevents root colonization, while jasmonic acid acts as an antagonist hormone and improves colonization.

Once the plant epidermis is reached by *Trichoderma* spp., a set of reactions is triggered to restrict fungal growth and invasion. On the other hand, fungi also produce and secrete molecules enabling them to tolerate the attack and remain inside of the root tissue.

Plants present an immune response triggered by the recognition of organisms, including microbes, which interact with them in the

rhizosphere or colonize their tissues. Plant immunity results in compatible or incompatible processes related to the microbe or plant species, and provides protection to invaders. In general, plants sense and respond to microbes by two main branches: pathogen/microbe-associated molecular pattern (PAMP/MAMP)-triggered immunity, PTI/MTI, or effector-triggered immunity (ETI) (Jones and Dangl, 2006). PTI/MTI is activated as a result of the interaction between pathogen/microbe-associated molecular patterns (PAMP/MAMP) and host pattern recognition receptors (PRRs). These include receptor-like kinases (RLKs) and receptor-like proteins (RLPs) (Monaghan and Zipfel, 2012). PAMP/MAMPs are common microbial compounds essential to survival, and include bacterial flagellin and fungal chitin. PTI is also triggered by damage-associated molecular patterns (DAMPs), which arise from damage caused by organism invasion into plant tissues (Boller and Felix, 2009) (see Fig. 4).

To overcome or inhibit this first line of defense, pathogens have evolved virulence effector molecules or effector proteins. Following this, there is a second line of plant defense ETI. ETI involves interaction between plant resistance R protein receptors, such as the nucleotide-binding domain leucine-rich repeat (NB-LRR) proteins, and cognate pathogen effector molecules that target PTI or other key host functions. In fact, pathogen effector molecules have evolved to minimize the plant immunity system, enabling their colonization by pathogens (Jones and Dangl, 2006).

PTI and ETI involve the activation of a mitogen-activated protein kinase (MAPK) cascade and WRKY transcription factors (TFs), coupled to a rapid calcium cytoplasmic influx and accumulation of reactive oxygen species (ROS). Additionally, there is callose deposition between the plant cell wall and plasma membrane at the site of infection. Kinase cascade activation results in increased synthesis of pathogenesis-related proteins and phytoalexins, as well as cell wall fortification and stomatal closure (Pitzschke et al., 2009). Despite the sharing of molecular events and results triggered during PTI-MTI/ETI, the latter is qualitatively stronger and faster, often leading to localized cell death (hypersensitive response-HR) (see Fig. 4).

Induced resistance in tissues distal from the infection site is one of the downstream effects of PTI/ETI, in which signals propagate to undamaged parts of the plant. This enhances their defense capacity, a well-described pathogen-induced resistance known as systemic acquired resistance (SAR) (Pieterse et al., 2014). SAR is characterized by salicylic acid accumulation, which can lead to aHR (Durrant and Dong, 2004) with the expression of genes coding for acidic PR proteins, mainly those with antimicrobial activity (Park and Wu, 2016; van Loon et al., 1998). Therefore, SAR is accompanied by the coordinated activation of pathogenesis-related genes (van Loon et al., 1998; Vernooij et al., 1994).

Beneficial microbes, such as plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungus (PGPF), also lead the host plants to a state of resistance. This is called induced systemic resistance (ISR), in which the entire host plant is protected on an enhanced level from future attacks by a broad spectrum of invaders upon local infection (Walters et al., 2013). This process is tightly regulated by a network of interconnected signaling pathways in which plant hormones play a central role, especially jasmonic acid (JA) (Bardoel et al., 2011).

Upregulation of pathogenesis-related (PR) genes is also associated with biosynthesis of JA and ethylene (ET). JA is known to be involved in biosynthesis of PR proteins and proteinase inhibitors. ET acts in synergy with JA signaling, with involvement in PR protein production and enhancement of the SA-mediated NPR1 pathway in SAR (Leon-Reyes et al., 2009; Lorenzo et al., 2003).

Plant growth-promoting fungi *Trichoderma* spp. modulate the aforementioned plant defense responses, leading to a coordinated

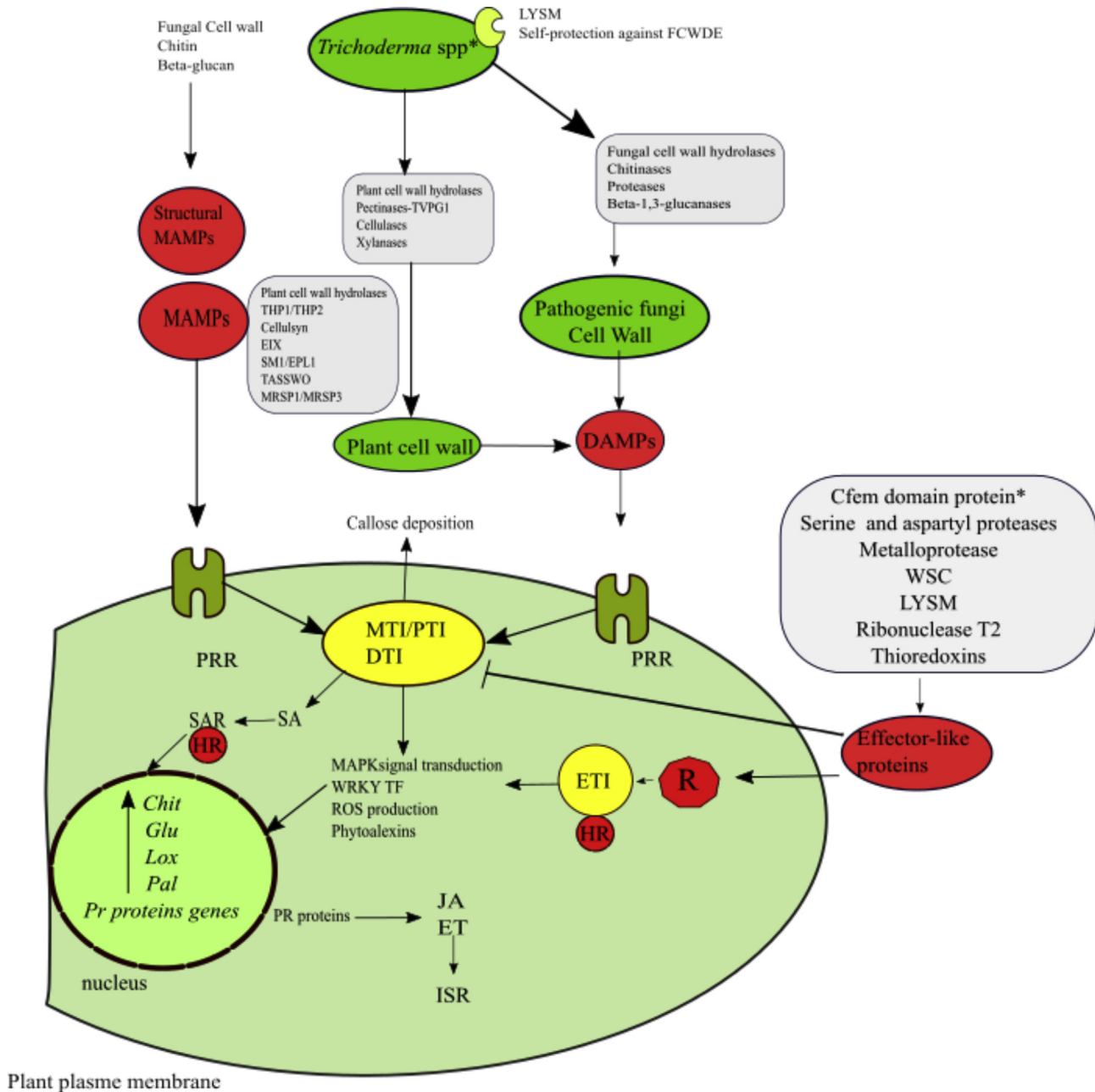


Fig. 4. A model summarizing proposed mechanisms involved on modulation of plant defense response by *Trichoderma* spp. MAMPs/PAMPs: pathogen/microbe-associated molecular patterns; DAMPs: damage-associated molecular patterns; PTI/MTI: Pathogen/Microbe-associated molecular pattern (PAMP/MAMP)-triggered immunity PRR: Pattern recognition receptors; ETI: Effector-triggered immunity; Systemic acquired resistance, SAR; ISR-Induced systemic resistance; HR: Hypersensitive response; SA: Salicylic acid; JA: Jasmonic acid; ET: Ethylene; R: Resistance R protein receptors.

transcriptomic, proteomic, and metabolomic response (Djonovic et al., 2007; Harman et al., 2004b; Pereira et al., 2014). Such changes have been described for associations between *T. virens*/Cotton, *T. harzianum* T22/*Zea mays*, *T. harzianum* T39/Grapevine, *T. harzianum* ALL 42/*P. vulgaris*, and *T. virens*/Maize and tomato (Mukherjee et al., 2012a; Pereira et al., 2014; Shores et al., 2010). The type of defense response triggered varies according to the *Trichoderma* sp., host plant, time after colonization, and inoculum concentration. There are records of the triggering of induced systemic resistance, system acquired resistance-like response, or both. Therefore, there is no classical model to describe the molecular events or type of defense response triggered by *Trichoderma* sp. in association with host plants. As previously described for

pathogenic fungi, chitin and β -glucans constituting *Trichoderma* spp. cell walls act as structural MAMPs (Hermosa et al., 2013). *Trichoderma* spp. also releases DAMPs by the action of fungal cell wall hydrolases (Chitinases, β -1,3-glucanases, and proteases) on pathogen cell walls, as well as by the action of plant cell wall hydrolases like pectinases, cellulases, and xylanases (Alkooranee et al., 2017; Hermosa et al., 2013). In addition to their role as producers of DAMPs, plant cell hydrolases have been described as modulators of host plant resistance against fungal phytopathogens, acting as MAMPs or through other mechanisms. Endopolygalacturonase TVPG2 from *T. virens*, previously described as an inducer of tomato resistance against *B. cinerea*, is quite related to the expression control of *tvpg1*, endopolygalacturonase 1-encoding gene *tvpg1*

(Sarrocco et al., 2017). *T. virens* endopolygalacturonase 2-encoding gene, *tvpg2*, showed a regulatory role in the induction of *tvpg1*, endopolygalacturonase 1-encoding gene, which encodes TVPG1. This was previously described as having a role in host plant root colonization and production of DAMPs. A *tvpg2*-knockout strain fails to transcribe the inducible *tvpg1* during *in vivo* interaction with tomato roots, significantly reducing its defense against *B. cinerea* (Sarrocco et al., 2017). Sarvanakumar et al. (2016) showed the role of two *T. harzianum* cellulase-like enzymes (THPH 1 and THPH 2) in maize-induced resistance (ISR) against *Curvularia* leaf spots, acting as an MAMP. A mixture of *T. virens* cellulases and cellusyn induces the biosynthesis of volatile compounds coupled to increased levels of endogenous JA in tobacco, lima bean, and corn (Piel et al., 1997). Melon cotyledons infiltrated with an active cellulase from *T. longibrachiatum* produced a rapid oxidative burst and the activation of early defense mechanisms associated with the ET and SA signaling pathways, remarkably increasing peroxidase and chitinase activity. In addition, its heat-denatured form induces ET production (Martinez et al., 2008). A β -1,4-endoxyranase (EIX) isolated from *T. viride* elicits plant defense responses, ISR, in tobacco (*Nicotiana tabacum* L.) and tomato cultivars, increasing ethylene biosynthesis independently of its hydrolytic activity (Sharon et al., 1993). Secreted small cysteine-rich proteins like SM1/EPL1, SM2, and expansin-like proteins such as swollenins also take part in the induction of the defense response. The carbohydrate-binding domain (CBD) from TASSWO is capable of stimulating defense responses in tomato plants, potentially acting as an MAMP. The protein SM1 (small protein-1) from *T. virens* and *T. harzianum*, and its homologous Epl1 (eliciting plant response-like) from *T. atroviride*, are non-enzymatic elicitors of ISR (Djonovic et al., 2007; Seidl et al., 2006). The role of SM1/EPL1 in *T. virens*, *T. atroviride*, and *T. harzianum* interactions with host plants present differences. *T. virens*, *T. atroviride* SM1, and EPL1 knockout mutants are unable to protect maize from the attack of *C. heterostrophus* (Lamdan et al., 2015). *T. harzianum* ALL 42 SM1 mutant instead shows the ability to trigger the expression level of defense related genes, *lox* and *glu*, in a more intense manner in comparison to the wild type (Gomes et al., 2015). A similar negative effect of small secreted cysteine-rich proteins was also described by Lamdan et al. (2015). The authors showed that *T. virens* SSCP knockout lines for two expansin-like proteins, MRSP1 and MRSP3, showed higher ISR-promoting activity than wild type (Lamdan et al., 2015) (see Fig. 4).

More recent finds reinforced and demonstrated the secretion and presence of genes encoding effector-like proteins in the genomes of symbiotic *Trichoderma* spp. These pathogen effectors can inhibit host plant defense response allowing their establishment on host plants (Guzmán-Guzmán et al., 2017; Kubicek et al., 2011; Mendoza-Mendoza et al., 2018). Therefore, the close and beneficial interaction between *Trichoderma* spp. and host plants is a final result of the balance between triggering and inhibition of defense, avoiding a strong defense response. This would lead to a hypersensitive response and ultimately, plant cell death. Serine and metalloproteases, thioredoxins, glycoside hydrolases, hydrophobins, proteins containing the domain common in fungal extracellular membranes (CFEM), LysM proteins, WSC domain proteins, ribonucleases T2, and eliciting plant response protein (EPL) are among the proteins identified as putative like-effectors (Guzmán-Guzmán et al., 2017; Mendoza-Mendoza et al., 2018).

The involvement of serine and metalloproteases as effectors has been described in pathosystems (Franceschetti et al., 2017). *F. oxysporum* f. sp. *lycopersicum* secretes a serine protease, Sep1, and a metalloprotease, Mep1, that act synergistically to cleave host chitinases. This prevents their activity in degrading fungal cell walls and producing DAMPs (Jashni et al., 2015). They are also a candidate for full virulence, since a double mutant of Sep1 and Mep1 showed

reduced disease on tomato plants (Jashni et al., 2015). An avirulence protein secreted by the rice blast fungus *M. oryzae* and homologous to other avirulence proteins from other organisms, AVR-Pita, presents typical features of zinc metalloproteases and catalysis (Giraldo et al., 2013; Orbach et al., 2000). Despite the detailed description of their role as effector proteins, protease activity has not been linked to their action to date.

In pathosystems, CFEM proteins have been described as cell-surface receptors, signal transducers, or adhesion molecules related to host plant–pathogen interactions and colonization (DeZwaan, 1999; Kulkarni et al., 2003). Regarding *Trichoderma* spp., Lamdan et al. (2015) showed decreasing on the abundance of a set of CFEM domain proteins during the interaction of *T. virens* and maize roots. In addition, *T. virens* knockout lines for these proteins showed higher ISR-promoting activity than wild type. However, the action mode of these proteins has not yet been elucidated.

Proteins containing the LysM motif might inhibit PTI-scavenging chitin oligomers liberated by the action of host plant PR proteins on fungal cell walls (Hermosa et al., 2013). It has also been suggested that LysM domains may provide fungi with a mechanism of self-protection against their own chitinases (Gruber et al., 2011).

The role of WSC domain proteins in interactions between beneficial fungi and host plants has not quite been established. For the beneficial fungus *Piriformospora indica* FGB1, a WSC domain protein was identified as suppressor of immunity in different plant hosts, altering fungal cell wall composition and properties (Rovenich et al., 2016; Wawra et al., 2016). These proteins also have been related to cellular resistance and cell wall perturbation, oxidation, high osmolarity, and metal ions (Tong et al., 2016). Ribonucleases classified as T2 family RNases have been described in genomes of other mycoparasitic *Trichoderma* spp. However, their function on interactions between *Trichoderma* and host plants has not been established. Thioredoxins may act by scavenging oxidative stress, a crucial strategy of resistance to allow the permanence of *Trichoderma* spp. in host root plant tissue, as previously suggested (Nogueira-Lopez et al., 2018).

Knowledge about the interaction of *Trichoderma* spp. with their hosts and their transcriptomic and proteomic approaches is a useful and powerful tool for describing an extensive list of protein candidates to play roles in these interactions. More efforts to perform function-oriented experiments are required to describe the action mode of the previously described proteins and their involvement in *Trichoderma*/host plant interactions. Many questions still remain to be answered in regard to this.

Promotion of plant growth caused by *Trichoderma* spp. can be a result of their indirect action, increasing the solubilization and availability of plant nutrients and micronutrients. It may also be a direct action for controlling the level of phytohormones, production of auxin or auxin-like effect molecules, and proteins which act by changing root architecture (Contreras-Cornejo et al., 2009; Hermosa et al., 2012; Nieto-Jacobo et al., 2017). Among the phytohormones, ethylene (ET) promotes root-hair initiation and elongation, but in contrast to auxin, ET inhibits lateral root formation and elongation.

Mutant strains of *T. harzianum* overexpressing the hydrophobin QID74 in association with cucumber leads to significantly longer lateral roots, as well as more numerous and longer secondary root hairs. These modifications increase the total absorptive surface, facilitating nutrient uptake and the translocation of nutrients in the shoots, ultimately resulting in increased total plant biomass (Samolski et al., 2012). The same kind of growth promotion was described for a hydrophobin from *T. longibrachiatum* in association with tomato and tobacco (Ruocco et al., 2015).

The major SMs produced by different *Trichoderma* strains, harzianolide and 6-pentyl- α -pyrone, also act as inducers of plant

growth presenting an auxin-like effect (Vinale et al., 2008). In addition to the former molecules, other volatile organic compounds, terpene derivatives, were recently added to the list of molecules secreted by *Trichoderma* spp. able to increase root surface and plant biomass (Lee et al., 2016).

The growth-promoting activity of *T. atroviride* and *T. asperellum* on tomato and canola seedlings has been suggested to be associated with the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (ACCD) (Contreras-Cornejo et al., 2009; Viterbo et al., 2010). ACCD activity reduces the level of ACC precursors for ET biosynthesis, decreasing the effects triggered by it. In the absence of ET, the effects of auxin produced by the fungus-ruled plant growth and root development. As discussed for the triggering of defense response, the molecular mechanism underlying growth promotion is still unclear.

6. The future of friendly microbes in crop production

6.1. Antimicrobial peptides (AMPs)

Higher organisms fight against a great variety of pathogens using several defense strategies, such as the production of antimicrobial peptides (AMPs), which have become an interesting tool to reduce crop losses (Pelegrini et al., 2011, 2008). AMPs are generally active against various kinds of infectious agents, being most effective as antibacterial agents, fungicides, antiviral agents, and antiparasitic agents, reducing the risk of resistance development in pathogenic microorganism populations. The difference in membrane architecture of prokaryotes and eukaryotes imparts microbial selectivity of AMPs, since AMPs are active at μM concentrations not generally toxic to cells of higher organisms (Perron et al., 2006). AMPs are small and low molecular weight peptides (generally consisting of 12–50 amino acids) and, unlike some antibiotics proteins, are normally synthesized through ribosomal protein synthesis machinery (Fox, 2013). The term “AMPs” is used for the peptides of eukaryotes, while “bacteriocins” is used for the bacterial defense peptides and proteins (Wiesner and Vilcinskas, 2010). AMPs are classified based on their structure and the presence of cysteine disulfide bond, which stabilize their structure. In plants, the main classes of AMPs are cyclotides, defensins, thionins, lipid transfer proteins, snakins, and hevein-like, vicilin-like, and knottins. Other AMPs include: IbAMPs, 2S albumin peptides, purindolines, hairpinins, β -barrelins, and glycine-rich cysteine-free peptides. The latter are unique in their amino acid composition and structure, and distinct from the aforementioned classes (Goyal and Mattoo, 2014). It is well known that most AMPs show hydrophobic regions with a net positive charge at physiological pH, and thus are commonly referred to as cationic AMPs. Plant AMPs with net negative charge are known as anionic AMPs. They therefore interact with the hydroxylated phospholipids, lipopolysaccharides, and teichoic acids in microbial membranes, which present negatively charged components. These support the inclusion of the peptides into the membranes, leading to permeabilization by pore formation in what is described as a detergent-like manner (“carpet” mechanism) (Brogden, 2005; Goyal and Mattoo, 2014). Concerning antifungal activity, Yokoyama et al. (2009) showed that AMP chitin-binding capability plays a crucial role in antifungal activity, and the antifungal mechanism may differ from the antibacterial mechanism (Van Der Weerden et al., 2010). The AMP antiviral effect depends on different factors, such as: direct interaction with the viral envelope, disrupting or destabilizing it; competition with viruses for the host membrane, preventing viral interaction with specific cellular receptors; and prevention of the expression of viral genes in the earlier infection stages, affecting propagation and viral infection (Salas et al., 2015). The structure of AMPs can alter its

activities, such as the linearization of cyclic antimicrobial peptides, which generally alters their ability to interact with cell membranes. Preliminary evidence has indicated that the structure maintained especially by the disulfide bonds is important to antimicrobial activity (Park et al., 1992; Tamamura et al., 1993). However, there is also evidence indicating that the function of amphipathic structure (α -helical or disulfide-linked β -sheet) and high cationic charge is the main feature for the biological activity of AMPs (Rao, 1999). The *in vitro* activity of many plant AMPs indicates potential utility in agribusiness. Thus, more than 2000 peptides are known and were cataloged in several databases available in the public domain, such as the Antimicrobial Peptide Database (<http://aps.unmc.edu/AP/main.php>) and others (Sarika et al., 2012). Furthermore, there are many works listing different classes of plant AMPs (De Souza Cândido et al., 2014; Goyal and Mattoo, 2014; Pelegrini et al., 2011; Salas et al., 2015). Plant genetic transformation with AMP sequences is a promising approach that combines broad-spectrum activity and efficient antibacterial mechanisms, and has been successfully implemented in different plant species (Boscariol et al., 2006; Osusky et al., 2005). Studies have shown that when heterologous, variant, synthetic, or other AMPs are introduced into plants, they present broad-spectrum resistance to diverse types of phytopathogens (Osusky et al., 2005, 2004; 2000; Ponti et al., 2003). Furman et al. (2013) evaluated the effect of constitutive expression of a dermaseptin coding sequence, a cationic AMP isolated from frogs of the *Phyllomedusa* genus. This exhibited *in vitro* activity against bacteria, filamentous fungi, protozoa, and yeast at micromolar levels, and did not show toxicity to human cells (Amiche and Galanth, 2011; Kastin, 2013; Mor et al., 1994) when expressed in sweet orange plants against *Xanthomonas* spp. The results showed a strong reduction in the frequency and intensity of citrus canker symptoms. Combined expression of antimicrobial transgenes could also be a suitable approach to obtain stable, broad-range protection against different kinds of phytopathogens. Rivero et al. (2012) combined different constructs expressing dermaseptin, lysozyme (which hydrolyze the N-acetyl-D-muramic acid: N-acetyl D-glucosamine linkage of peptidoglycans), and AP24 (a thaumatin-like pathogenesis-related protein belonging to the PR-5 family) coding sequences in potato (*Solanum tuberosum*) plants, and reported high levels of resistance to different species of bacteria and fungi. Besides broad-range protection, AMPs are also associated with other physiological aspects of plants. Nahirñak et al. (2012) reported that overexpression of the AMP *snaking-1*-encoding gene in potato plants enhanced resistance to *R. solani* and *Erwinia carotovora* pathogens. However, when this gene was silenced, it was found to affect growth and development processes such as cell division, primary metabolism, and cell wall chemistry. Goyal et al. (2013) described the construction of a new gene called *msrA3* by the molecular engineering of the N terminus of the *temporin A* gene, which belongs to a family of smallest antimicrobial peptides in nature. Using plant transformants for this gene, the authors tested transgenic potato lines for responses to abiotic stress and resistance to the potato pathogen *F. solani*. They showed that *msrA3* expression modulated the physiology and gene transcript profiles of the transgenic potato plants. Their results suggested that MSRA3 regulates the common step(s) of the hypersensitive (HR) and reactive oxygen species (ROS) defense pathways. Although some reports indicated that most naturally occurring AMPs exhibit a narrow activity spectrum, low activity against important pathogens, or high toxicity against human and plant cells (Bechinger and Lohner, 2006; Marcos et al., 2008), the major barrier for the use of AMPs as antibiotics is their toxicity or ability to lyse eukaryotic cells. This is normally expressed as hemolytic activity, or toxicity to human red blood cells. The selective toxicity is due to the fact that in eukaryotic cell membranes, the phospholipids which are

negatively charged are predominantly in the inner leaflet of the lipid bilayer, while the outer leaflet is mainly composed of cholesterol and sphingomyelin, which present no charge or electrically neutral (zwitterionic) (Matsuzaki, 1999). Hemolysis of eukaryotic cells requires the peptides to insert into the hydrophobic core of the membrane, perpendicular to the membrane surface, and interaction of the nonpolar face of the amphipathic α -helix with the hydrophobic lipid core of the bilayer. The peptide may thus form transmembrane channels/pores and the hydrophilic surfaces point inward, producing an aqueous pore (“barrel-stave” mechanism) (Jiang et al., 2008). Modern agriculture requires the use of antimicrobial compounds with low toxicity and reduced negative environmental impact. Thus, the development and design of new molecules by the use of combinatorial-chemistry procedures coupled to high-throughput screening systems are interesting avenues. Since the characteristics of the AMPs determine their “modus operandi”, direct modification of these features allows the design of new and specific AMPs. The modification of natural sequences seems to be a promising strategy for *de novo* synthesized peptides, using chemical libraries oriented for this purpose (Montesinos and Bardají, 2008). Zeitler et al. (2013) used this strategy for the development of structurally different groups of peptides. Four groups were assayed for their ability to inhibit bacterial growth and fungal spore germination, being highly active at different concentrations with no hemolytic activity at concentrations up to 200 mg/ml. These new molecules were also active after spraying on the plant surface.

6.2. Pathogen-derived resistance (PDR)

Besides the growing demand for food with every passing year, one of the greatest constraints affecting agricultural productivity is the impact of phytopathogens (Agrios, 2005). These include viruses, which are responsible for a significant number of commercially relevant plant diseases. With limited effective countermeasures, this places them among the most important agricultural pathogens (Gómez et al., 2009). Genetic engineering and biotechnology offer opportunities for disease prevention, such as the introduction of genes from diverse sources into plants. This could generate disease resistance, with none of the species barriers that apply to conventional strategies. The expression of structural viral nucleic acid sequences (e.g. coat protein, movement protein, or replicase protein genes) in plants is known as pathogen-derived resistance (PDR). This is a concept first proposed by Sanford and Johnston (1985), and generally offers a broader range of resistance to the related viruses. The technique is effective against a low level of inoculum, but as with most viral proteins, this strategy can produce elicitors of R gene-driven effector triggered immunity (ETI). This may cause HR (for review see Garcia and Pallás, 2015), which is out of the scope of this review. On the other hand, the accumulation of non-structural viral nucleic acid sequences can bring about protection, by introducing a transgenic RNA to cause degradation of the transcripts or genomic RNA of plant viruses. Although resistance is highly species-specific, it is effective against a high level of inoculum (Galvez et al., 2014; Koh et al., 2014). Viral genes expressing for intact or modified replicase protein or RNA-dependent RNA polymerase (RdRp) were used, and were reported to provide protection (Baulcombe, 1996; Lomonosoff, 1995). Evidence indicated that different mechanisms may be responsible for replicase-mediated resistance to different virus species. However, most of the reports describe resistance mediated by a functional or dysfunctional protein, interfering with the replicase enzyme complex and disrupting the viral replication cycle. It was also proposed that protein produced by the transgenic plants somehow interferes with the function of the replicase produced by the virus. It may

potentially bind to host factors or virus proteins that regulate replication and virus gene expression (Beachy, 1997). This type of resistance is often confused with RNA-mediated resistance. This mechanism is related to post-transcriptional gene silencing in transgenic plants, and resistance is dependent upon the sequence similarity between the sense RNA products of the transgene and the inoculated virus (Marano and Baulcombe, 1998; Tenllado et al., 1996). The major secondary metabolites produced by different *Trichoderma* strains, harzianolide and 6-pentyl- α -pyrone, also act as inducers of plant growth, presenting an auxin-like effect (Vinale et al., 2008). In addition to the former molecules, volatile organic compounds named terpene derivatives were recently added to the list of molecules secreted by *Trichoderma* spp. that are able to increase root surface and plant biomass (Lee et al., 2016).

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Fungal diseases also impact crop production worldwide, and their control is mainly carried using chemical fungicides. This is efficient, but if applied on a large scale can cause a remarkable impact on the environment. Within this context, the exploitation of biological systems to overcome disease occurrence is a useful and harmless alternative strategy for improving crop production. *Trichoderma* spp. are already used in formulations directly applied on soils to control plant pathogens, especially phytopathogenic fungus. Due to their action as antagonists of plant pathogens, they also can be used as biofertilizers and bioprotectors, improving plant health and resistance to diseases, respectively.

The successful use of *Trichoderma* isolates to trigger the desired positive effects occurs depending on their ability to adapt to the biome in which it will be introduced, in terms of abiotic and/or biotic stresses. Therefore, there is a greater chance of success when using isolates obtained from the same biome into which it will be re-introduced.

The function of symbiotic fungus may be improved by the development of mutants with increased activity. Mutants may be obtained using random genetic modification or site-direct mutagenesis, aiming for the knockout or over-expression of a gene. Genome editing using the CRISPR/Cas9 system has emerged as a powerful tool that facilitates genetic alteration in a variety of organisms. Despite this, there is not yet any record of the use of this method to obtain symbiotic *Trichoderma* spp. genomic editing. On the other hand, *Trichoderma* spp. genes may be used to develop transgenic plants with increased health and ability to resist to abiotic or biotic stresses.

There are published papers showing the improvement of plant protection by expressing the genes of antimicrobial proteins from *Trichoderma* spp., as well as genes related to resistance to biotic stresses. However, commercial cultivars are not yet available.

6.3. Plantibodies

Hiatt et al. (1989) first showed that individual cDNAs for immunoglobulin κ - and γ -chains could be efficiently expressed in plants to form and assemble functional antibodies, later named “plantibodies” (De Jaeger et al., 2000). Plantibodies have been developed for the following purposes: therapeutic applications (Fischer et al., 2003); immunomodulations (the expression of

antibodies to disrupt the function of antigens, or inhibit the activity of a host enzyme or metabolite that may be involved in the infection process) (Jobling et al., 2003); and for the protection of plants, which could be designed to target any pathogen, sequestering the antigens which are often required to complete the infection cycle and thus preventing disease (for review see Safarnejad et al., 2011). Furthermore, the plantibodies approach has several advantages over PDR in transgenic plants, which may create more virulent pathogens via genetic recombination (Aaziz and Tepfer, 1999). There are also some limitations, including: most antibodies cannot form their critical disulfide form in the reducing environment of the cytoplasm, affecting its secondary structure and thus, its function; difficulties targeting the antibodies to subcellular organelles; and only a few antibodies bind to the active sites of enzymes, and thus they generally do not neutralize enzyme function. Stable antibody fragments (Fv) have therefore been engineered by connecting the domains with a hydrophilic and flexible peptide linker to create single-chain Fv fragments (scFvs) (Huston et al., 1988). These molecules are particularly suitable for expression in plants. This is because of their small size (25 kDa) compared to full-size immunoglobulins (150 kDa) and their lack of assembly requirements. Alternatively, serum of camels, dromedaries, and llamas contains a unique type of antibody destitute of light chains, thus called heavy-chain antibodies (HCAb) (Hamers-Casterman et al., 1993). These have a lower molecular weight (12–15 kDa) than scFv and conventional antibodies, binding their antigen by one single domain. This is the variable domain of the heavy immunoglobulin chain, thus referred to as (VHH) or 'nanobody' (Muyldermans, 2001) (Muyldermans, 2001). They display resistance to heat, detergents, and high concentrations of urea, as they do not require disulfide bonds for stability and thus are appropriate for expression in plants (Jobling et al., 2003). The heavy-chain antibodies target correctly to subcellular organelles and inhibit the enzyme function in plants more efficiently than antisense approaches. Furthermore, the development of the phage display approach (which permit selection of peptides and proteins, including antibodies, with high affinity and specificity for nearly any target) (Krishnaswamy et al., 2009), and the generation of synthetic scFv libraries have greatly improved the applicability of these strategies (Prins et al., 2008). This has been observed for the cytoplasmic expression of an scFv antibody against the coat protein of artichoke mottled crinkle virus in transgenic tobacco (*Nicotiana benthamiana*), reducing the viral infection and delaying progression of disease symptoms (Tavladoraki et al., 1993). Cervera et al. (2010) reported virus resistance in the transgenic Mexican lime plants, which expressed two different scFv constructs against epitopes of the major *Citrus tristeza* virus. Interestingly, Boonrod et al. (2004) reported a strategy to achieve virus resistance based on the expression of scFvs against a conserved domain in a plant viral RNA-dependent RNA polymerase (RdRp), which is essential for replication of the viral genome. This strategy showed to be effective in the inhibition of complementary RNA synthesis of different plant virus RdRps *in vitro* and virus replication *in planta*. Furthermore, transgenic lines of *N. benthamiana* expressing different scFvs in different cellular compartments (cytosol or endoplasmic reticulum) showed varying degrees of resistance against four plant viruses from different genera. The authors also described that the scFvs had specific affinity to a distantly related human hepatitis C virus in *in vitro* assays, indicating the use of anti-RdRp-scFvs beyond plant pathology. Alternatively, Ghannam et al. (2015) reported a novel genetic approach for plant virus resistance based on the *in planta* expression of camelid-specific nanobodies against broad bean mottle virus. For fungal pathogens, Peschen et al. (2004) reported the expression of fusion proteins consisting of a *Fusarium*-specific recombinant antibody linked to antifungal peptides. The results

indicated inhibition of fungal growth and high levels of protection against *F. oxysporum* f.sp. *matthiolae* in transgenic *Arabidopsis thaliana* plants. Yajima et al. (2010) reported transgenic canola (*Brassica napus*) lines expressing *S. sclerotiorum*-specific scFv antibody, which showed significant levels of tolerance against stem rot. Mollicutes are bacteria that can infect humans, animals, and plants (Bové, 1993). These pathogens have lost the genes responsible for the synthesis of a bacterial cell wall (regressive evolution). Thus, they are limited by a single cytoplasmic membrane. Their metabolism and growth can be inhibited by antibodies directed against their membrane epitopes, and thus mollicutes may be an ideal candidate for a plantibody-controlled resistance strategy. This was reported by Le Gall et al. (1998), who engineered, cloned, and expressed a functional scFv-specific fragment recognizing the major immunodominant membrane protein of stolbur phytoplasma in *Escherichia coli* (to confirm its specificity and stability), and thus conferred resistance in tobacco plants. Antibody-based detection assays are commercially available for *Xylella fastidiosa*, a member of the gamma proteobacteria which causes a variety of diseases on a wide range of economically important crops, including grape and citrus. They are effective at the species level, but not at the subspecies level. Yuan et al. (2015) used phage display technology to successfully develop a library of scFv antibody fragments, which have the potential to distinguish *X. fastidiosa* at the subspecies level.

The use of these approaches in plant protection, animal treatment, or even in human medicine may offer new and safer alternatives to control microbial diseases. Their unique properties allow the use of different molecules from various sources against diverse pathogens, representing promising future candidates for combating microbial diseases. This will allow avoidance of the development of drug resistance, thus reducing losses and increasing the yield, quality, and safety of agricultural products. Furthermore, other interesting biological activities and potential applications, such as signaling molecules, immune modulators, antitumor agents, drug delivery vehicles, and plant transgenes mediators, can be developed.

7. Conclusion

Food security is a large global issue that causes risk to human health. However, in order to guarantee food security, it is necessary to monitor crops throughout planting, pre-harvest, and post-harvest. The use of the fungus *Trichoderma* is promising, as this fungus can interact directly with the plant, promoting growth and providing the crops with resistance to a pathogenic fungus. This will lead to an increase in yield and food production. On the other hand, fungi of the genus *Trichoderma* can attack phytopathogenic fungi, by different mechanisms. Knowledge of the biological processes of the interactions between *Trichoderma*/plants/pathogens is fundamentally important for the development of specific strategies for each culture, with the aim of promoting food security. In addition, with the advent of molecular biotechnology, the molecules produced by microorganisms, including fungi of the genus *Trichoderma*, can be identified to immunize plants against diseases. The use of recombinant DNA technology for plant research may also aid in combating disease and adverse environmental effects. It is our hope that the technology contained in this review can be applied gradually as an alternative to the use of agrochemicals, for more sustainable and food-fed agriculture.

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