RESEARCH REVIEW

INTERNATIONAL MICROBIOLOGY (2013) 16:69-80
doi:10.2436/20.1501.01.181 ISSN 1139-6709 **www.im.microbios.org**

INTERNATIONAL MICROBIOLOGY
OPEN ACCESS

The contribution of *Trichoderma* to balancing the costs of plant growth and defense

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Received 25 March 2013 · Accepted 14 June 2013

Summary. *Trichoderma* is a fungal genus of cosmopolitan distribution and high biotechnological value, with several species currently used as biological control agents. Additionally, the enzyme systems of the fungus are widely applied in industry. Species of *Trichoderma* protect plants against the attack of soil-borne plant pathogens by competing for nutrients and inhibiting or killing plant pathogenic fungi and oomycetes, through the production of antibiotics and/or hydrolytic enzymes. In addition to the role of *Trichoderma* spp. as biocontrol agents, they have other beneficial effects on plants, including the stimulation of plant defenses and the promotion of plant growth. In this review, we focus on the complex plant defense signaling network that allows the recognition of fungi as non-hostile microbes, including microbial-associated molecular patterns (MAMPs), damage-associated molecular patterns (DAMPs) and secreted elicitors. We also examine how fungal interactions with plant receptors can activate induced resistance by priming and balancing plant defense and growth responses. Our observations are integrated into a model describing *Trichoderma*-plant hormone signaling network interactions. [**Int Microbiol** 2013; 16(2):69-80]

Keywords: *Trichoderma* spp. · plant–*Trichoderma* symbiosis · *Arabidopsis thaliana* · phytohormone networking

Introduction

Trichoderma spp. are non-pathogenic soil-borne (free-living) fungi that colonize the roots of many plants as opportunistic, avirulent plant symbionts [18]. In addition, these fungi have been exploited in biotechnological applications and provide important benefits to agriculture, such as their ability to protect crops against disease and increase crop yield under field conditions [29]. Most species of *Trichoderma* have been linked to biocontrol against plant pathogenic fungi, oomycetes, and even nematodes [37]. The versatility of *Trichoderma* strains in suppressing pathogen-induced diseases and their broad environmental opportunism have been reported [4,15]. Several *Trichoderma* strains are included as active biofungicide matter in registered biological control substances, although many *Trichoderma* formulations for commercial use against plant pathogens are not registered as plant protection products. Instead, they may be marketed as plant inoculants or plant strengthening agents, which for the respective manufacturers circumvents the time and expense necessary for product registration [19]. Other beneficial effects of *Trichoderma* spp. in plants are their promotion of plant growth, their ability to elicit plant defenses against pathogen attack and environmental stress, and their use in the improvement or maintenance of soil productivity [20,57].

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The mycotrophic lifestyle of *Trichoderma*, which includes mycoparasitism and saprophytic growth, has been reviewed in the context of a comparative analysis of the genomes available for this genus [15]. The genomes of *Trichoderma virens* and *Trichoderma atroviride* encode the highest number of chitinolytic enzymes of all fungi described. Furthermore, proteases are also expanded in these two genomes, supporting the hypothesis that protein degradation is a major trait of mycoparasites. Indeed, *T. virens* has the highest number of non-ribosomal peptide synthetases compared to other fungi; these enzymes are involved in the formation of secondary metabolites with antifungal activity. Moreover, the saprophytic behavior of *Trichoderma* is evidenced by its large set of carbohydrate-active enzymes, produced for the extracellular digestion of dead or decayed organic matter as a food source [25]. By contrast, *Trichoderma* genomes encode relatively few enzymes that catalyze the breakdown of pectin, the cement of plant cell walls, indicating a special relationship with living plants.

As rhizosphere colonizers, *Trichoderma* spp. have developed opportunistic mechanisms for their adaptation to abiotic stresses as well as for nutrient uptake and solute transport. In the plant, these processes are facilitated by the induction of cell wall extension and expansion, secondary root development, lateral root hair production and a higher photosynthetic rate [10,52,57]. This cross-talk between *Trichoderma* and plants plays a role in phytohormone signaling [20]. Previous *International Microbiology* publications have described the biotechnological use of *Trichoderma* strains, proteins and genes [37] and have reviewed the biocontrol mechanisms of these fungi [4]. In this third *Trichoderma* article, we attempt to explain how the *Trichoderma*–plant interaction modifies the equilibrium between plant growth and defense against both pathogens and environmental damage.

Microbial recognition by plants

Unlike many animals, plants lack mobile defender cells and an adaptive somatic immune system. Instead, they rely on the innate immunity of each cell and on systemic signals emanating from sites of infection [22]. However, plants have an amazing capacity to recognize pathogens through strategies involving both conserved and variable pathogen elicitors. These compounds are secreted by attackers as virulence effector molecules to manipulate plant defensive responses [14]. Conversely, a primary plant immune response has evolved that allows plants to recognize the common features of organisms that interact with them and to translate this recognition into a defense response that is specifically directed against the invader encountered [22]. Like insects and vertebrates, plants have receptors for microbe-associated molecules and respond to many of the same molecules that animals respond to, including bacterial lipopolysaccharide, flagellin, and the translation elongation factor EF-Tu [2]. Plants are also responsive to a wide variety of molecules associated with fungi and oomycetes, including chitin, β-glucan and ergosterol [60]. Pathogenic and symbiotic microbes can produce signaling molecules that share several characteristics but induce contrasting responses in plants: either immunity (rejection) or symbiosis (acceptance). Perception of these molecules by the plant involves similar receptors, raising the question of how friends are distinguished from foes such that the response to these signals is the correct one.

Plant immune responses to invading microbes comprise at least two recognition systems and sets of receptors. In innate plant immunity, early basal defenses to limit the attacker's growth are initiated on the external face of the host cell, where conserved microbial elicitors, called microbial- or pathogen-associated molecular patterns (MAMPs or PAMPs), are recognized by pattern recognition receptors (PRRs) located in the plasma membrane [5]. PRRs are subdivided into two main groups: receptor kinases, which are also able to recognize abiotic stresses, and the less abundant receptor-like proteins [14]. Similarly, plants respond to endogenous molecules released by pathogen invasion, such as cell wall oligomers or cuticular fragments, referred to as damage-associated molecular patterns (DAMPs). Stimulation of PRRs leads to a signal transduction cascade and the activation of MAMP- or PAMP-triggered immunity (MTI or PTI).

Along with their primary innate immune response, plants can activate another line of defense, referred to as induced resistance. This type of resistance often acts systemically throughout the plant and is typically effective against a broad spectrum of attackers. However, plants have to balance the costs and potential benefits of investing in defenses since these are ecologically costly. Indeed, natural selection is presumed to have favored the evolution of inducibility, meaning that these defenses are only produced in the presence of attackers [61]. Nonetheless, successful pathogens deliver virulence molecules (avr proteins, coronatine, gibberellins, etc.), called effectors, that suppress PTI responses, facilitating colonization and causing disease. In this so-called effector-triggered susceptibility (ETS) [22], pathogens manage to suppress defense responses through the deployment of effector molecules. Conversely, plants can sense these effectors by dominant intracellular plant resistance gene products. Of these, the most abundant are a

class of polymorphic intracellular receptors containing a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) domain, so-called nucleotide-binding domain leucine-rich (NBS-LRR) proteins [60]. Pathogen effectors from diverse kingdoms are recognized by NBS-LRR proteins and activate different signal transduction pathways, depending on the organism interacting with the plant. NBS-LRR plasticity can be explained by the fact that these proteins have the highest frequency of major effect mutations and differentially methylated regions, at least in the genome of *Arabidopsis* [53]. Thus, an epigenetic control may limit their expression until released from silencing by pathogen attack. NBS-LRR-mediated disease resistance is effective against pathogens that grow only on living host tissue (obligate biotrophs: e.g., *Ustilago maydis, Cladosporium fulvum*) or that are hemibiotrophic (e.g., *Pseudomonas syringae, Colletotrichum acutatum*) but not against pathogens that kill host tissue during colonization (necrotrophs: e.g., *Botrytis cinerea, Sclerotinia sclerotiorum*). This recognition induces effector-triggered immunity (ETI). The quantitative output of the plant immune system has been illustrated in a zigzag model [22], in which the final amplitude of disease resistance or susceptibility is proportional to [PTI – ETS + ETI].

Plant defense signaling networks

Generally, PTI/MTI and ETI give rise to similar responses: a rapid influx of calcium ions from external stores, a burst of reactive oxygen species (ROS), activation of mitogen-activated protein kinases (MAPKs), reprogramming of gene expression and the deposition of callosic cell wall appositions at sites of attempted infection [47]. However, ETI is qualitatively stronger and faster than PTI/MTI and often involves a form of localized cell death called the hypersensitive response, which arrests the growth of biotrophic fungi. PTI is generally effective against non-adapted pathogens, conferring so-called nonhost resistance, whereas ETI is active against adapted pathogens [14].

Through a phosphorelay mechanism, MAPK signaling cascades link upstream receptors to downstream targets and are involved in the regulation of plant development, growth, programmed cell death, and the responses to a diversity of environmental stimuli including cold, heat, ROS, UV light, drought, and pathogen attack. MAPK cascade activation by PTI/MTI or ETI culminates in the expression of defense genes and, as a result, in the synthesis of certain pathogenesis-related proteins (glucanases, chitinases) and of the phytoalexin camalexin, as well as cell wall fortification and stomatal closure [47]. Together, these modifications confer resistance to pathogens, as phytohormones regulate the production of downstream defense molecules.

The importance of salicylic acid (SA), jasmonates (JA, jasmonic acid and the volatile methyl jasmonate) and ethylene (ET) as primary signals in the regulation of the plant immune response is well established [44]. Although there are exceptions, biotrophic pathogens are generally sensitive to the defense responses regulated by SA, which is required for local resistance and for a type of systemic resistance known as systemic acquired resistance (SAR). By contrast, pathogens with a necrotrophic lifestyle are commonly deterred by defenses controlled by JA and ET, which act as signal transduction molecules for a systemic resistance pathway that is referred as induced systemic resistance (ISR). ISR requires the ankyrin repeat-containing protein NPR1, a key regulator in SA signaling [45].

However, plants are often subjected to simultaneous or subsequent invasions by multiple attackers and beneficial microbes, which can influence the primary induced defense response of the host plant. Activation of different plant defense mechanisms implies costs in ecological fitness. Crosstalk between the SA-JA pathways often results in their reciprocal antagonism, which has been interpreted as an adaptive, cost-saving strategy because the different enemies are susceptible to different defense strategies [61]. SA-JA antagonism is widespread across plants but these have evolved mechanisms to respond in different ways or with different intensities to similar stimuli. For example, in *Arabidopsis* SA is typically prioritized over JA. Plants also use ET as a third component to fine-tune defenses by prioritizing JA over SA induction in response to multiple attackers [26].

Root signaling and beneficial microbes

Beneficial relationships between plants and microbes often occur in the rhizosphere and improve plant growth or help the plant to overcome biotic or abiotic stresses [70]. For example, the roots of *Arabidopsis* respond to different MAMPs in a tissue-specific manner and MTI signaling in the roots is very similar to that observed in the leaves [36]. To establish mutualistic interactions with the host plant, beneficial microbes minimize stimulation of its immune system, which is triggered locally in the roots upon MAMP perception [70]. Beneficial interactions between the plant's roots and microbes [rhizobia, plant-growth-promoting rhizobacteria (PGPR), mycorrhizae

and plant-growth-promoting fungi (PGPF)] initially elicit a MTI response, which is subsequently suppressed by the production of effector/elicitor molecules.

A common feature of the resistance responses induced by beneficial microbes is priming, or the JA-dependent activation of plant defenses prior to contact with a challenging microbe, in which plants respond either faster, more strongly, or both to pathogen attack [9]. Priming may be initiated in response to an environmental cue that reliably indicates an increased probability of encountering a biotic stress or by the colonization of a beneficial microbe, but a primed state may also persist as a residual effect following an initial exposure to the stress. For example, the typical pathogen-induced hypersensitive response is often induced with greater efficiency in plants that have previously undergone pathogen attack or were subject to a beneficial microbe. Primed plants do not express costly defense responses; rather, since priming gives rise to defense activation only upon recognition of a potential intruder, it is also an ideal mechanism to control interactions with invading beneficial microbes [70]. Priming is the plant's solution to the dilemma posed by the trade-off between disease protection and the costs involved in defense activation.

Because beneficial microbes are also recognized as alien organisms, active interference with the plant's immune signaling network is fundamental to the establishment of intimate mutual relationships. In many cases, SA, JA and ET have interconnected pathways and emerge as the dominant regulators in this process [44]. In *Arabidopsis*, SA mediates a change in the cellular redox potential that leads to SAR activation through the NPR1 regulator and TGA transcription factors [16]. Cytosolic NPR1 plays a key role in the crosstalk between SA and JA and is involved in JA/ET-dependent ISR [59].

The phytohormone ET is a gas recognized by plasma membrane receptors that negatively regulate ET responses. In response to ET perception, the receptors engage in downstream signaling of positive regulators that activate the expression of ET-responsive genes. ET and JA cooperate in inducing ET response factor 1, a transcription factor that drives the activation of defense-related genes and positively regulates the expression of JA-inducible genes involved in defense responses [28].

JA signaling is controlled by a complex formed by the E3 ubiquitin ligase SCF^{COI1} complex and jasmonate ZIM-domain (JAZ) proteins. The latter act as JA receptors and, at low JA levels, are transcriptional repressors of JA-responsive gene expression. When the JA concentration is elevated, JAZ proteins are degraded in the 26S proteasome, leading to the rapid activation of a multitude of JA responses [23]. Upon JAZ degradation, the versatile transcription factor MYC2 is released to promote JA-induced gene expression. MYC2 is repressed by JAZ proteins and positively regulates JA-responsive genes but negatively regulates JA/ET-responsive genes. MYC2 contributes to the repression of early MTI responses.

Rhizobia are sensitive to SA-regulated defense responses [42], and SA signaling negatively affects root mycorrhization [27]. The colonization of *Arabidopsis* roots by the PGPF *Piriformospora indica* is also limited by SA-dependent defenses [21]. Accordingly, beneficial microbes have evolved mechanisms to efficiently suppress the SA-triggered responses of host plants and thus to establish successful infections. In contrast to pathogen-induced SAR, which is dependent upon SA signaling and associated with the enhanced expression of a large set of pathogenesis-related genes, PGPR- and PGPF-triggered ISR is often SA-independent and not associated with major changes in defense-related gene expression [63]. In its interactions with *Arabidopsis*, *P. syringae*, generally considered to be a leaf pathogen but also a root colonizer, and *P. indica* take on the JA signaling pathway to suppress both early- and late-activated defense responses, suggesting that JA pathway activation is a common strategy of bacteria and fungi that is designed to affect host immunity in the roots [36]. MYC2 has been proposed as a priming regulator for enhanced JA-responsive gene expression during PGPR-mediated ISR [48]. The early signaling steps of *Trichoderma* and PGPR-mediated ISR also require activation of the transcription factor MYB72 in the roots [55], although the combined application of *Trichoderma* and PGPRs has not resulted in enhanced disease control [1].

Beneficial fungi and bacteria also have to suppress ET signaling, to avoid being recognized as uninvited guests. In this context, MYC2 also regulates crosstalk between JA signaling pathways and those of other phytohormones such as abscisic acid (ABA), gibberellins (GAs), and auxins (indole acetic acid, IAA), and is involved in JA-regulated plant growth and development [23]. Many PGPR and PGPF are able to produce IAA or GAs [10,17,58] that attenuate the relative strength of the interaction between SA signaling and phytohormone networking [44]. GAs also stimulate growth by promoting the destruction of a set of plant growth repressor proteins (DELLAs) that promote susceptibility to necrotrophs and resistance to virulent biotrophs [40]. DELLAs permit the flexible and appropriate modulation of plant growth in response to changes in the environment [23].

Lateral root and plant development are supported by IAA, although, at high concentrations, IAA inhibits growth. IAA is recognized by a SCF-type E3 ligase receptor similar to that of

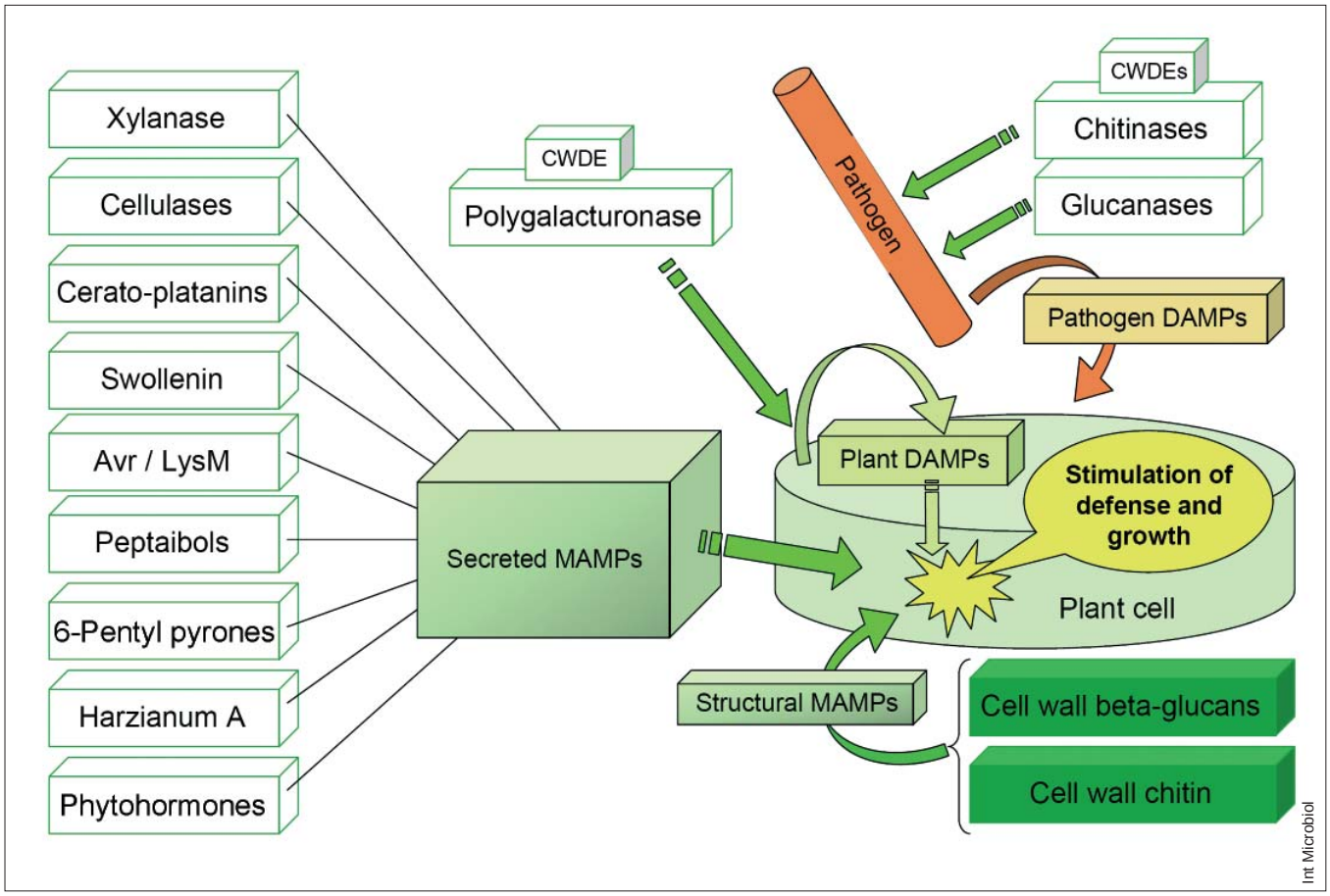

Fig. 1. Structural and secreted *Trichoderma* microbial-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs), resulting from the activity of the *Trichoderma* cell wall degrading enzyme (CWDE) on pathogens or plants, such that, in the latter, defense or growth responses are elicited.

JA and has a reciprocal antagonism with SA [44]. Beneficial microbes such as *Azospirillum* and *Trichoderma* produce IAA, facilitating root development and colonization and helping to overcome MTI. IAA and ET regulation of DELLA restraint are key steps in our *Trichoderma*-plant crosstalk model, described in a previous review [20], to explain the integrated plant development and immunity responses activated by this fungus.

Trichoderma and plant responses

The capacities for *Trichoderma* opportunism have enabled species such as *T. hamatum* to live both as common components of the soil and rhizosphere and as endophytes. The presence of potential fungal prey and plant root-derived nutrients in the rhizosphere may have driven ancestral *Trichoderma* spp. to colonize plant roots, facilitating the evolution of further positive interactions between *Trichoderma* and plants [15]. To establish a dialogue with plants (Fig. 1), *Trichoderma* releases DAMPs and expresses a collection of MAMPs and elicitors that, after recognition by NBS-LRR proteins—which are up-regulated in plants challenged with *Trichoderma* [33,56]—are able to induce different types of beneficial responses. In the following, we provide ten illustrative examples of these responses, induced by polygalacturonases, xylanases, cellulases, cerato-platanins, swollenins, avirulence proteins and lysin motif (LysM) domains, peptaibols, 6-pentyl pyrones, trichothecenes and phytohormones.

Polygalacturonase *ThPG1*. The *Thpg1* gene was detected in a transcriptome and subsequent proteome analysis using a three-component system (*Trichoderma harzianum*–tomato plantlets–pathogen [*Rhizoctonia solani* or *Pythium ultimum*]). The endopolygalacturonase ThPG1 is a plant cell-wall-degrading enzyme required for efficient root colonization by *T. har-*

zianum [38]. ThPG1 hydrolyzes plant pectin and produces oligogalacturonides that act as DAMPs when they are recognized by the PRR receptor wall-associated kinase-1 [12]. Recognition results in the activated production in the plant of polygalacturonase inhibitor protein. However, ThPG1 is unaffected by an inhibitor from *Arabidopsis* and is counteracted only by doses of bean polygalacturonase inhibitor proteins higher than those active against polygalacturonases from phytopathogenic fungi [38]. The down regulation of: (i) genes encoding two glycine-rich proteins (GRP19 and ATA20), which regulate wall-associated kinase-1 function linked to a SA-dependent defense response [41], (ii) two SA-responsive genes (*chs, Ltp*) and (iii) five root-strength and root-hair development genes (*xth9, xtr7, cys2, grp19* and *ata20*), detected in the aerial parts of *Arabidopsis* plants colonized with a *Thpg1*-silenced transformant, relates ThPG1 to SAR activation and the modification of cell wall structure and root architecture.

The *chs* gene encodes a chalcone synthase that is an important type III polyketide synthase of the phenylpropanoid pathway, whose expression causes the accumulation of flavonoid and isoflavonoid phytoalexins. This pathway is also involved in the SA defense pathway. Lipid transfer proteins show some structural similarities to the small secreted cysteine-rich proteins (SSCPs) of fungi and oomycetes. In addition, they bind lipid molecules of plasma membrane receptors and play a role in long-distance signaling during SAR. *Ltp* gene expression is involved in the *Trichoderma*-induced defense against *Phytophthora* spp. in pepper [3] and against *P. syringae* in *Arabidopsis* [8]. The *xth9* and *xtr7*genes encode a xyloglucan endotransglycosylase related to cell-wall loosening, the elongation of root tissue, and root hair initiation, and a xyloglucosyl transferase involved in root hair growth, respectively. The phytocystatin CYS2 is a membrane protein located in trichomes and in the guard cells of young leaves. As a potent inhibitor of cysteine proteases,CYS2 is expressed in roots after their physical damage, thus playing roles in plant development and stress responses. In addition, as a pathogenesis-related protein, it is involved in programmed cell death and defense mechanisms against insects and pathogens. GRP19 and ATA20 are wall-associated kinase-1 interactors involved in cell elongation, secondary cell wall formation, lignin biosynthesis and/or the deposition and establishment of innate immunity.

Xylanase Eix/Xyn2. The first recognized *Trichoderma* MAMP was an ET-inducing xylanase (Eix) produced by *T. viride* as a potent elicitor of the hypersensitive response and other plant defense mechanisms in some tobacco and tomato cultivars; this effect was independent of its xylan degradation activity. ET-inducing xilanase is recognized by the tomato PRRs LeEix1 and LeEix2, which span the plasma membrane and have extracellular LRR domains [49]. It elicits ET biosynthesis and then ISR in leaves by inducing expression of the gene encoding 1-aminocyclopropane-1-carboxylic acid (ACC) synthase [35]. A different xylanase, expressed by *T. reesei*, produces cell death in tobacco leaves, accompanied by typical defense responses that include an oxidative burst and the expression of defense genes following the activation of a specific cellular signal-transduction cascade. In the latter, an influx of extracellular Ca^{2+} ions is thought to be crucial for induction of the hypersensitive response [67].

Cellulases. A complex mixture of *Trichoderma* cellulases raises the level of endogenous JA after 30 min, followed by the transient emission of ET after 2–3 h when applied to cut petioles of tobacco, lima bean and corn [43]. In a later study [34], infiltration of an active cellulase from *T. longibrachiatum* into melon cotyledons produced a rapid oxidative burst and the activation of early defense mechanisms associated with the ET and SA signaling pathways, leading to a strong increase in peroxidase and chitinase activities. However, the infiltration of heat-denatured cellulase also induced ET but without the accumulation of SA or the promotion of a hypersensitive-like response. In both studies, the ET pathway was activated, demonstrating the role of ET in fine-tuning plant defenses.

Multiple systemic responses may derive from the action of several *Trichoderma* elicitors, inducing resistance via different parallel signaling pathways. Although rice root colonization by *T. asperellum* is associated with a clear SAR signaling cascade and suppression of the disease symptoms caused by *P. syringae* on leaves, application of culture filtrates of this fungus leads to the induction of both SA and JA/ET defense genes, probably because more than one elicitor triggers plant defenses [69]. The induction of plant responses by *Trichoderma* is a time- and concentration-dependent process. During the first hours of interaction, a reaction similar to SAR may occur as a result of *Trichoderma* colonization, when the fungus is applied to plant roots at high concentrations (10^7 conidia/ml). Although an increase in SA and JA occurs in the first hours of root colonization, SA and JA peak levels depend on the concentration of *Trichoderma* [54].

Cerato-platanins Sm1/Epl1. Cerato-platanins are hydrophobin-like SSCPs [Sm1 from *T. virens* and Epl1 from *T. atroviride* (*Hypocrea atroviridis*)] that accumulate in hyphae during

root colonization. These compounds act as elicitors of JA-induced ISR, but not SAR, in cotton and maize [20]. The abundance of SSCPs in the genomes of the biocontrol fungi *T. virens* and *T. atroviride* [25] is indicative of rhizosphere competence, although neither deletion nor overexpression of *Sm1* alters the phenotypic traits of the fungus or its efficiency in colonizing maize roots [13]. In any case, there is a striking level of diversity between the three species of *Trichoderma* sequenced to date, suggesting the rapid evolution of SSCPs.

Swollenin carbohydrate-binding domain. *Swo1* was identified in *T. reesei* as a novel gene with sequence similarity to plant expansins. It encodes a swollenin protein whose N-terminal carbohydrate-binding domain has a cellulose-binding function and a C-terminal expansin-like domain. In *T. asperellum*, a role in the ability to colonize cucumber roots within 6 h after inoculation has been described for this protein. The carbohydrate-binding domain of swollenin does not seem to be involved in an increase in ISR defense but it does have MAMP activity, since a synthetic 36-mer carbohydrate-binding domain peptide stimulates local defense responses in cucumber roots and leaves and affords local protection against *Botrytis cinerea* and *Pseudomonas syringae* infections [7].

The avr4 and avr9 avirulence protein homologues and LysM domains. In a proteome analysis of three-partner interactions between *T. atroviride* and the bean pathogens *Rhizoctonia solani* or *B. cinerea*, SSCP homologues of the avirulence proteins avr4 and avr9 from the tomato biotrophic fungus *Cladosporium fulvum* were identified in *T. atroviride* [33] and *T. harzianum* [18]. The binding of Avr4 to chitin has been confirmed experimentally. This avirulence protein is thought to shield the fungal cell wall from plant chitinases [60]. Avr9 is a PAMP with structural, but not functional, homology to carboxypeptidase inhibitors strongly induced under nitrogen limitation; it is not required for full virulence. The recognition of Avr4 and Avr9 leads to activation of the enhanced disease susceptibility 1 (*EDS1*) gene, encoding a NBS-LRR receptor, and to SA accumulation upon pathogen infection [60]. Through activation of SA, *EDS1* represses JA/ET defenses. In *Trichoderma–Arabidopsis* interactions [39], there is a strong down-regulation of SA-related genes, such as *EDS1, PR-1, chs* and *Ltp*, in leaves after only 4 h of *T. harzianum* inoculation; this response persists at 24 h, with an increase in the expression of these plant genes after 48 h. The activated disease resistance 1 gene, encoding a NBS-LRR receptor that regulates SA accumulation after an oxidative burst, is also down-regulated in leaves after 24 h of interaction, indicating a reduction in SA-dependent MTI responses after *Trichoderma* colonization. Several JA-related genes are likewise down-regulated at 24 h post-inoculation of *Trichoderma*; their expression levels are similar to those determined in *Arabidopsis* plants not inoculated with this fungus at 48 h. This suggests that plant defenses mediated by JA and SA are transiently reduced. Since *T. harzianum* is not perceived as hostile by the host plant, it is able to colonize the plant's roots. A cyclic behavior of SA and JA/ET responses induced by *Trichoderma* explains the different SAR or ISR responses described in the literature. It also supports the reduction in *Arabidopsis* leaf damage by the simultaneous accumulation of SA and JA/ET defense-related gene transcripts, detected at 96 h post-inoculation of *Arabidopsis* roots with *T. atroviride* [51].

Genes encoding chitin-binding LysM proteins are expanded in *Trichoderma* genomes [25]. These proteins suppress host defenses by sequestering chitin oligosaccharides, which act as elicitors of defense responses in plants subsequent to their recognition by the LysM-receptor kinases of the plasma membrane [11]. Scavenging of such oligomers is fundamental to the ability of the fungus to attack its hosts. As a mechanism for perceiving chitin, plants have likely evolved chitinases to release the active polymers from the cell walls of invading fungi, thereby triggering defense responses. LysM proteins most probably do not protect chitinous fungi against plant chitinases, but are more likely to be involved in the scavenging of chitin fragments that are released from fungal cell walls during infection, preventing them from acting as PAMPs that trigger PTI [6,11]. *Trichoderma* chitinases with LysM-binding modules are able to sequester chitin and thereby dampen plant defenses while facilitating root colonization. However, as biocontrol agents, if chitinous pathogens are present in the system, the mycotrophic activity of *Trichoderma* chitinases can also release chito-oligosaccharides from chitin substrates and the cell walls of their fungal targets, which contributes to the induction of defense.

Peptaibols. Peptaibols are peptides 5–20 amino-acids-long and composed of 2-aminoisobutyric acid and other non-proteinogenic amino acids. They are produced as secondary metabolites with antibiotic activity against fungi and bacteria. The 20-mer peptaibol alamethicin produced by *Trichoderma* induces the biosynthesis of volatile organic compounds in lima bean, principally via the JA-signaling pathway, but it also up-regulates SA biosynthesis [20]. This increase in SA reduces the production of JA-dependent volatile organic compounds, to not only modify plant defense strategies against

pathogens but also to control above-ground herbivorous insects and attract their natural enemies (parasitoids and predators) as well as pollinators [46].

Trichokonins are 20-mer peptaibols detected in *Trichoderma pseudokoningii*. They are able, locally and systemically, to induce ROS production and the accumulation of phenolic compounds at the application site in tobacco plants. Other activities include the induction of multiple systemic resistance against tobacco mosaic virus, through the up-regulation of SA, JA and ET signaling genes [30].

The 18-mer peptaibols of *T. virens* can elicit systemic defense responses in cucumber against the leaf pathogen *P. syringae*. The SA and JA pathways are induced after 48 h, although fungal or peptaibol challenge elicits a higher expression of the SA gene marker phenylalanine ammonia lyase and the JA gene marker hydroperoxide lyase, respectively [66]. In interactions with cucumber roots, *T. asperellum* induces both SAR and ISR responses to control *P. syringae*. In the former, phenylalanine ammonia lyase gene expression is detected in leaves 24 h post-inoculation, with a maximum reached at 48 h. ISR responses are evidenced by hydroperoxide lyase gene expression in the leaves 24–48 h after root inoculation [68]. Note that the *lox1* gene, which encodes a lipoxygenase involved in JA biosynthesis, is also induced in cucumber 1 h after root elicitation with *Trichoderma*, indicating a rapid local activation of ISR defense that coincides with the start of callose deposition.

6-Pentyl pyrones. The volatile pyrone 6-pentyl-2*H*-pyran-2-one (6PP) is a common *Trichoderma* metabolite responsible for the coconut aroma and yellow pigmentation associated with strains of this fungus. 6PP also inhibits the growth of pathogens such as *Fusarium oxysporum* [50]. At low concentrations, it regulates plant growth, including the production of more extensive and developed root systems, significantly increases plant height and leaf area, and increases seed germination and seedling height. Together, these effects suggest that 6PP acts as an auxin-like compound and/or as an auxin inducer [50]. A reduction in disease symptoms on tomato and canola seedlings treated with the purified metabolites 3 h before inoculation with, respectively, the pathogens *B. cinerea* and *Leptosphaeria maculans* is observed when 6PP is applied at 1 mg/l. Similar results have been obtained with higher concentrations of the butenolide harzianolide and the pyridine harzianopyridone. The *PR-1* gene was induced by 6PP and harzianopyridone at 1 mg/l in canola cotyledons, indicating the activation of a SA-dependent SAR response. At the same time, a chitinase *PR-3* gene related to JA-dependent defense was induced by the same amount of 6PP, harzianopyridone or azaphilone [64]. These are further examples of the multiple systemic defense responses induced by *Trichoderma*.

Trichothecenes. Sesquiterpenes are C_{15} terpene compounds that include trichothecenes, which are important mycotoxins known mainly for their phytotoxicity and for their toxic effects on animals and humans. T-2 mycotoxin, a biological weapon produced by *Fusarium* spp., is a problem in agriculture and animal feed because of its phytotoxicity. Trichothecenes are synthesized by *Trichoderma* species of the clade *Brevicompactum*. Thus, *T. brevicompactum* produces trichodermin, a phytotoxic compound that enables this species to be used as a biocontrol agent [62]. *Trichoderma arundinaceum* produces harzianum A, a trichothecene with antifungal activity against *B. cinerea* and *R. solani* [31] that elicits systemic defense and priming responses in tomato plants [32]. In the antagonistic interaction of *T. arundianceum* and *B. cinerea*, the former produces harzianum A while the latter inhibits the expression of genes in the trichothecene biosynthetic cluster. *B. cinerea* on tomato activates a typical JA response in the plant; *T. arundianceum* on tomato activates the expression of SA and JA signaling genes by the plant. In the interaction between *T. arundianceum*, *B. cinerea* and tomato, there is a dramatic increase in the expression of tomato plant defense-related genes belonging to the SA and JA pathways, compared to a background of *B. cinerea*-tomato and *T. arundianceum*-tomato conditions [32].

Phytohormones. *Trichoderma* promotes growth responses in plants. In the colonization of cucumber roots by *T. asperellum*, the fungus enhances the availability of P and Fe to the plant, leading to significant increases in its dry weight, shoot length and leaf area [57]. The cysteine-rich cell wall protein QID74 of *T. harzianum* modifies root architecture, increasing the total absorptive surface and facilitating nutrient uptake and the translocation of nutrients in the shoots, resulting in increased plant biomass through an efficient use of N, P, K and micronutrients [52]. An association between plant growth-promoting activities and reduced ET production has been suggested, based on a decrease in its precursor ACC through the microbial degradation of IAA in the rhizosphere and/or through microbial ACC deaminase (ACCD) activity. *Trichoderma* produces IAA and ACCD [10,65] and thereby manipulates the phytohormone regulatory network.

Support for our crosstalk model between the host plant and *Trichoderma* derives from the cross-communication between

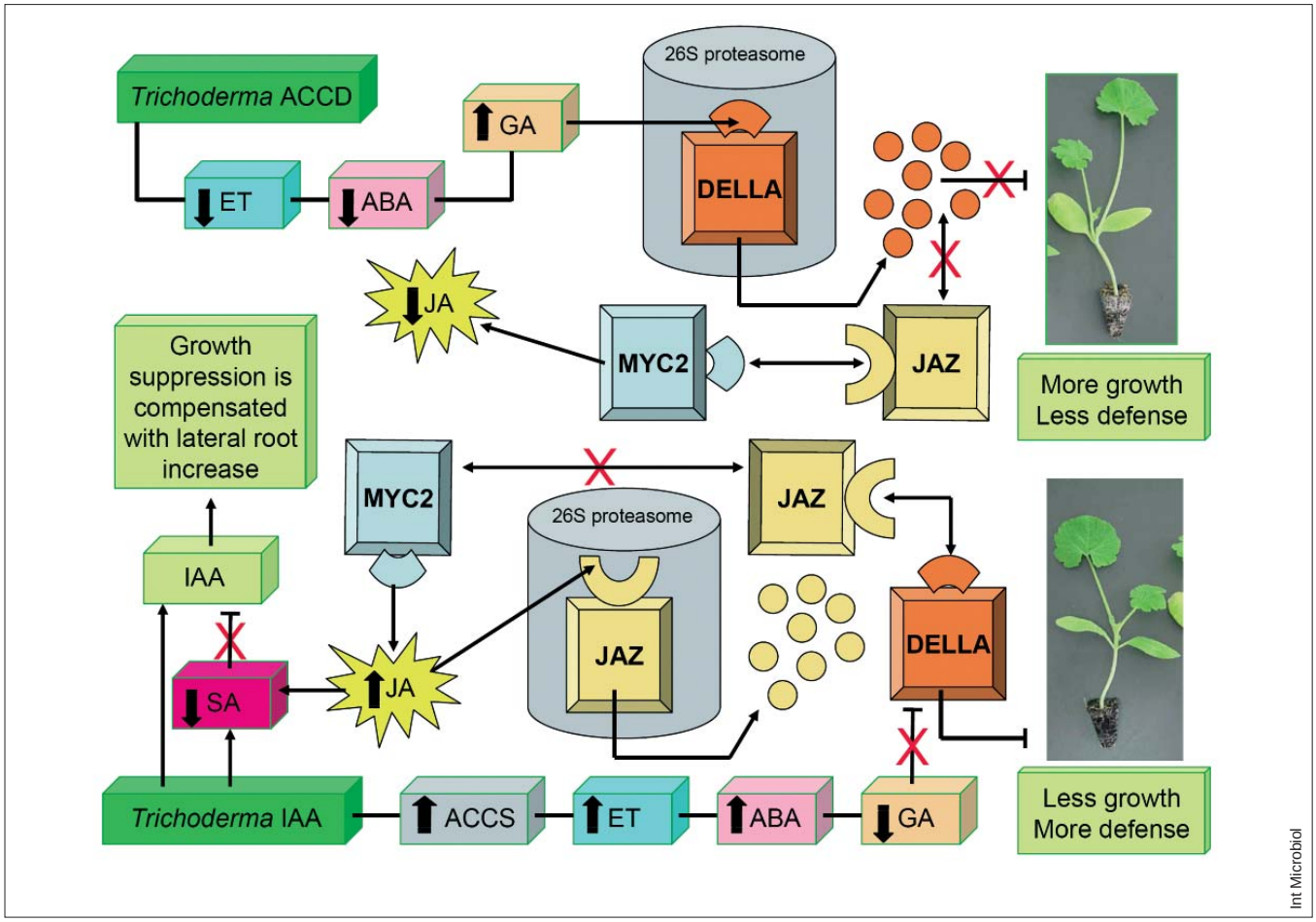

Fig. 2. Hypothetical *Trichoderma* interactions with the complex hormone signaling network of its host plant. *Trichoderma* 1-aminocyclopropane-1-caboxylic acid (ACC) deaminase (ACCD) production reduces plant ethylene (ET) signaling, leading to a reduction of abscisic acid (ABA) and an increase of gibberellin acid (GA) levels. As a result, plant growth is promoted, by degradation of DELLA proteins in the 26S proteasome, and plant defenses are suppressed, through the repression of the transcriptional activity of MYC2 transcription factor by binding to jasmonate ZIM-domain (JAZ) proteins. *Trichoderma* indole-3-acetic acid (IAA) production stimulates ET biosynthesis via ACC synthase (ACCS) in the plant. In turn, an increase in ABA biosynthesis is triggered, together with a reduction of GA levels, leading to reduced targeting to DELLA proteins and the repression of plant growth. DELLAs compete with MYC2 for JAZ binding, contributing rapidly to the activation of jasmonic acid (JA) signaling and defenses. *Trichoderma* IAA also contributes to antagonizing salicylic acid (SA) signaling in the plant and to compensating for growth suppression by the induction of lateral root development.

SA, JA and ET, and IAA and the response pathways of other hormones (GA and ABA) [20]. Briefly, ACCD activity reduces the availability of the ACC necessary for ET biosynthesis, which in turn promotes plant growth and suppresses defenses via GA signaling by increasing the degradation of DELLA proteins. In roots, the biosynthesis of ET and IAA is reciprocally regulated. The IAA produced by *Trichoderma* contributes to plant growth, lateral root development and to exogenous auxin-stimulated ET biosynthesis via ACC synthase. The latter in turn triggers an increase in ABA biosynthesis, commonly associated with plant development and abiotic stress defense, but also with callose priming and the regulation of defense gene expression through the activation of JA biosynthesis [44].

As noted above (Fig. 2), a model explaining the balance between defense and growth based on mutually antagonistic crosstalk between GA-JA signaling and DELLA-JAZ was recently proposed by Kazan and Manners [23]. In their model, growth promotion and defense suppression occur when, under suitable growth conditions, GA stimulates growth by promoting the destruction of DELLAs while defense is blocked through the repression of MYC2 transcriptional activity by (MYC2) binding to JAZ proteins. By contrast, there is growth suppression and defense activation when the plant is challenged by an attacker, e.g. a PGPF, and the JA-mediated degradation of JAZ proteins facilitates MYC2 transcriptional activity. This rapidly contributes to activating JA signaling, lea-

ding to further growth suppression by a reduction in DELLA degradation and tipping the balance towards defense.

The DELLA-JAZ model [23] is also compatible with the *Trichoderma*-plant interaction model proposed in this review (Fig. 2). During the transient period of reduced defenses triggered by *Trichoderma*, colonization of the intercellular spaces of the epidermis and cortex is permitted, plants have not a need to reduce their growth (*Trichoderma* enhances growth in *Arabidopsis* regardless of ISR inducibility [20]), while several genes related to ABA, IAA and abiotic stress responses [39], and amino-acid biosynthesis [8] are up-regulated.

Similarly, proteins involved in ROS scavenging, the stress response, terpene and ET biosynthesis, photosynthesis, photorespiration and carbohydrate metabolism are differentially regulated in cotyledons after cucumber root colonization by *Trichoderma* [54]. During a short colonization period, the GA-JA signaling interaction is likely to reach equilibrium, characterized by low levels of defense gene expression, because of the competition between DELLAs and MYC2 for JAZ binding (Fig. 2). Interestingly, the versatile MYC2 also regulates interactions between JA signaling and light, phytochrome signaling, and the circadian clock [24].

Final remarks

Trichoderma spp. broad environmental opportunism has facilitated their activity in the rhizophere, with beneficial effects on plants, including the stimulation of plant defenses and plant growth. These abilities support the application of *Trichoderma* strains as plant inoculants or plant-strengthening agents in agriculture and forestry. *Trichoderma* has evolved to interact with plants such that it is not perceived as an enemy. Its structural and secreted proteins and secondary metabolites act as MAMPs, while its enzymes, by acting against other fungi and plant cell walls, generate DAMPs. MAMPs and DAMPs can be recognized by specific plant receptors that activate signaling cascades, leading to defense responses and the activation of a phytohormone networking that cross-communicates defense responses, against pathogen attack and environmental stress, as well as growth signaling pathways. *Trichoderma* uses a transient period in which plant defenses mediated by JA and SA are reduced to colonize the roots of its host. The plant, conversely, limits *Trichoderma* growth to the intercellular spaces of the cortex and epidermis. Expression of the defense-related genes of the JA/ET and/or SA pathways may overlap, depending on many factors [20], although *Trichoderma* can induce a faster and stronger JA-dependent systemic response (priming) in other parts of the plant. In addition to the contribution of *Trichoderma* to plant growth and defenses by producing the correct concentrations of ET and IAA, the mutually antagonistic crosstalk interactions between GA-JA signaling and the DELLA-JAZ balance, regulated by MYC2, have been proposed as switching elements to resolve the plant's conflict between investing in defense or growth.

Acknowledgements. The authors thank to all those working on *Trichoderma*-plant interactions for improving our knowledge of this complex system. Funding was obtained from the Junta de Castilla y León (SA260A11-2 and LE125A12-2), Junta de Andalucía (AGR 6082) and the Spanish Ministry of Science and Innovation (AGL2009-13431-C02 and AGL2012-40041-C02).

Competing interest. None declared.

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