Mini-Review

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Plant-beneficial effects of *Trichoderma* and of its genes

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Trichoderma (teleomorph *Hypocrea*) is a fungal genus found in many ecosystems. *Trichoderma* spp. can reduce the severity of plant diseases by inhibiting plant pathogens in the soil through their highly potent antagonistic and mycoparasitic activity. Moreover, as revealed by research in recent decades, some *Trichoderma* strains can interact directly with roots, increasing plant growth potential, resistance to disease and tolerance to abiotic stresses. This mini-review summarizes the main findings concerning the *Trichoderma*–plant interaction, the molecular dialogue between the two organisms, and the dramatic changes induced by the beneficial fungus in the plant. Efforts to enhance plant resistance and tolerance to a broad range of stresses by expressing *Trichoderma* genes in the plant genome are also addressed.

Introduction

The need for increasing agricultural productivity and quality has led to an excessive use of chemical fertilizers, creating serious environmental pollution. The use of biofertilizers and biopesticides is an alternative for sustaining high production with low ecological impact. Different soil-borne bacteria and fungi are able to colonize plant roots and may have beneficial effects on the plant. Besides the classic mycorrhizal fungi and *Rhizobium* bacteria, other plant-growth-promoting rhizobacteria (PGPR) and fungi such as *Trichoderma* spp. and *Piriformospora indica* can stimulate plant growth by suppressing plant diseases (Van Wees *et al.*, 2008). These micro-organisms can form endophytic associations and interact with other microbes in the rhizosphere, thereby influencing disease protection, plant growth and yield. The plant–microbe association involves molecular recognition between the two partners through a signalling network mediated by the plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). JA and ET have been described as signal transduction molecules for induced systemic resistance (ISR) due to the effect of beneficial microbes, and the signal transduction pathway through SA accumulation is found in the systemic acquired resistance (SAR) induced by attack by pathogens. A common feature of ISR responses to beneficial

microbes is priming for enhanced defence. In primed plants, defence responses are not activated directly, but are accelerated upon attack by pathogens or insects, resulting in faster and stronger resistance to the attacker encountered (Van Wees *et al.*, 2008).

Trichoderma (teleomorph *Hypocrea*) is a fungal genus found in many ecosystems. Some strains have the ability to reduce the severity of plant diseases by inhibiting plant pathogens, mainly in the soil or on plant roots, through their high antagonistic and mycoparasitic potential (Viterbo & Horwitz, 2010). The recent comparative genome sequence analysis of two recognized biocontrol species – *Trichoderma atroviride* and *Trichoderma virens* – has afforded us a better understanding of how mycoparasitism arose in a common *Trichoderma* ancestor as a lifestyle of the genus (Kubicek *et al.*, 2011). The presence of fungal prey and the availability of root-derived nutrients may have been major attractors for the ancestors of *Trichoderma* to establish themselves in the rhizosphere and to facilitate the evolution of positive interactions with plants (Druzhinina *et al.*, 2011). The control of a broad range of plant pathogens, including fungi, oomycetes, bacteria and viral diseases, through elicitation by *Trichoderma* of ISR or localized resistance has been reported (Harman *et al.*, 2004). Some *Trichoderma* rhizosphere-competent strains have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defences against biotic and abiotic damage (Shores *et al.*, 2010). In recent years, an increasing number of studies have contributed to unravelling the molecular basis of the

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; ACCD, ACC deaminase; ET, ethylene; IAA, indole-3-acetic acid; ISR, induced systemic resistance; JA, jasmonic acid; MAMP, microbe-associated molecular pattern; PGPR, plant-growth-promoting rhizobacteria; ROS, reactive oxygen species; SA, salicylic acid; SAR, systemic acquired resistance; SSCP, small secreted cysteine-rich protein.

plant–*Trichoderma* dialogue and the beneficial effects of *Trichoderma* to plants.

In this short review we shall try to summarize the main findings on the direct *Trichoderma*–plant interaction (Fig. 1) and the efforts undertaken to enhance plant resistance and tolerance to a broad range of stresses by expressing *Trichoderma* genes in the plant genome.

***Trichoderma* spp. can colonize root intercellular spaces**

Trichoderma strains are found in many root ecosystems. Similarly to the situation with mycorrhizae, the highly hydrated polysaccharides of the root-secreted mucigel layer and the mono- and disaccharides excreted by plant roots into the rhizosphere encourage growth of the fungi. It has been observed that plant-derived sucrose is an important resource provided to *Trichoderma* cells to facilitate root colonization, the coordination of defence mechanisms, and increased rate of leaf photosynthesis (Vargas *et al.*, 2009). Solute transporters such as a di/tripeptide transporter and a permease/intracellular invertase system involved in the acquisition of root exudates have been described in *Trichoderma* (Vizcaino *et al.*, 2006; Vargas *et al.*, 2009).

Strains able to promote plant growth and provide protection against infections must be able to colonize plant roots. Colonization involves an ability to recognize and adhere to roots, penetrate the plant, and withstand toxic metabolites produced by the plant in response to invasion. In *Trichoderma*, adherence to the root surface can be mediated by hydrophobins, which are small hydrophobic proteins of the outermost cell wall layer that coat the fungal cell surface, and expansin-like proteins related to cell wall development. *Trichoderma asperellum* produces the class I hydrophobin TasHyd1, which has been shown to support the colonization of plant roots, possibly by enhancing its attachment to the root surface and protecting the hyphal tips from plant defence compounds (Viterbo & Chet, 2006), and the swollenin TasSwo, an expansin-like protein with a cellulose-binding domain able to recognize cellulose and modify the plant cell wall architecture, facilitating root colonization (Brotman *et al.*, 2008). Plant cell-wall-degrading enzymes are also involved in active root colonization, as occurs with the endopolygalacturonase ThPG1 from *Trichoderma harzianum* (Morán-Díez *et al.*, 2009).

Seventy-two hours after root colonization, *Trichoderma* yeast-like cells were observed together with a strengthening

Fig. 1. Schematic representation of *Trichoderma*–plant molecular signalling and plant-induced effects. T, *Trichoderma*; P, pathogen; IAA, indole-3-acetic acid; ACCD, ACC deaminase; ET, ethylene; JA, jasmonic acid; SA, salicylic acid; ISR, induced systemic resistance.

of plant epidermal and cortical cell walls and the deposition of newly formed barriers containing large amounts of callose and infiltrations of cellulose (Chacón *et al.*, 2007). Typical host reactions to *Trichoderma* were found beyond the sites of potential fungal penetration and, unlike *P. indica* colonization, which does not induce plant cell wall reinforcement, callose-enriched wall appositions were apparently efficient in the restriction of fungal growth to the intercellular spaces of the epidermis and cortex, preventing the entry of *Trichoderma* into the vascular stele (Yedidia *et al.*, 1999). Plants also react against fungal invasion by synthesizing and accumulating antimicrobial compounds. The ability to colonize plant roots depends strongly on the capacity of each strain to tolerate them. In *Trichoderma*, this resistance has been associated with the presence of ABC transport systems, which are key factors in the multiple interactions established by *Trichoderma* biocontrol strains with other microbes in a potentially toxic or antagonistic environment (Ruocco *et al.*, 2009), with rapid degradation of the phenolic compounds exuded from plants (Chen *et al.*, 2011), and with the suppression of phytoalexin production, as detected in *Lotus japonicus* during colonization with *Trichoderma koningii* (Masunaka *et al.*, 2011). In a proteome analysis, a small secreted cysteine-rich protein (SSCP) was identified in *T. harzianum*

and *T. atroviride*, proving to be a homologue of the avirulence protein Avr4 from *Cladosporium fulvum* (Harman *et al.*, 2004). It has been proposed that the binding of Avr4 to chitin could protect *Trichoderma* against plant chitinases (Stergiopoulos & de Wit, 2009).

Induction of plant defences by *Trichoderma*

In addition to pre-formed physical and chemical barriers, plants have an immune system that is able to detect motifs or domains with conserved structural traits typical of entire classes of microbes but not present in their host, namely the pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs, respectively). MAMP-triggered plant responses are elicited rapidly and transiently. Early MAMP responses involve ion fluxes across the plasma membrane, the generation of reactive oxygen species (ROS), nitric oxide, ET and also, but later, the deposition of callose and the synthesis of antimicrobial compounds. Many MAMPs have been identified for PGPR, such as flagellin or lipopolysaccharides, but also secreted compounds including antibiotics, biosurfactants and volatile organic compounds have been shown to elicit systemic resistance. Effective *Trichoderma* strains produce a variety of MAMPs (Table 1), which to date are those most widely described among

Table 1. *Trichoderma* MAMPs currently identified in different species

MAMP/effector	<i>Trichoderma</i> species	Activity	Reference
Proteins			
Xylanase Xyn2/Eix	<i>T. viride</i>	A xylanase that elicits ET biosynthesis and hypersensitive response in tobacco leaf tissues	Rotblat <i>et al.</i> (2002)
Cellulases	<i>T. longibrachiatum</i>	Activated and heat-denatured cellulases elicit melon defences through the activation of the SA and ET signalling pathways, respectively	Martínez <i>et al.</i> (2001)
Cerato-platanins Sm1/Epl1	<i>T. virens</i> / <i>T. atroviride</i>	Hydrophobin-like SSCP orthologues that can induce expression of defence responses in cotton and maize	Djonović <i>et al.</i> (2006), Seidl <i>et al.</i> (2006)
Swollenin TasSwo	<i>T. asperelloides</i>	Expansin-like protein with a cellulose-binding domain capable of stimulating local defence responses in cucumber roots and affording local protection against <i>B. cinerea</i> and <i>P. syringae</i>	Brotman <i>et al.</i> (2008)
Endopolygalacturonase ThPG1	<i>T. harzianum</i>	Involved in active colonization of tomato root and ISR-like defence in <i>Arabidopsis</i>	Morán-Díez <i>et al.</i> (2009)
Secondary metabolites			
Alamethicin (20mer peptaibol)	<i>T. viride</i>	Elicitation of JA and SA biosynthesis in lima bean	Engelberth <i>et al.</i> (2001)
Trichokonin (20mer peptaibol)	<i>T. pseudokoningii</i>	Induces the production of ROS, the accumulation of phenolic compounds at the application site and virus resistance in tobacco plants through multiple defence signalling pathways	Luo <i>et al.</i> (2010)
18mer peptaibols	<i>T. virens</i>	Elicitation of cucumber systemic defences against <i>P. syringae</i>	Viterbo <i>et al.</i> (2007)
6-Pentyl- α -pyrone, harzianolide and harzianopyridone	Various	Low-concentration metabolite activating plant defence mechanisms and regulating plant growth in pea, tomato and canola	Vinale <i>et al.</i> (2008)

plant-beneficial fungi (Lorito *et al.*, 2010). The first recognized *Trichoderma* MAMP was identified as an ET-inducing xylanase (Xyn2/Eix), produced by *Trichoderma* as a potent elicitor of plant defence responses in specific tobacco and tomato cultivars (Rotblat *et al.*, 2002). The Eix epitope recognized by plants consists of five surface-exposed amino acids that are not involved in enzyme activity. *Trichoderma*-activated and heat-denatured cellulases also elicit melon defences through the activation of the SA and ET signalling pathways respectively (Martínez *et al.*, 2001). Some *Trichoderma* proteins involved in root colonization can also act as MAMPs. Swollenin TasSwo (Brotman *et al.*, 2008) stimulates defence responses in cucumber roots and leaves and affords local protection against fungi and bacteria, and the endopolygalacturonase ThPG1 (Morán-Díez *et al.*, 2009) generates a response in *Arabidopsis* similar to the ISR triggered by PGPR. Orthologues of the SSCP cerato-platanin family – Sm1 from *T. virens* and Epl1 from *T. atroviride* – are accumulated in the hyphae during root colonization and act as MAMPs in cotton and maize (Djonović *et al.*, 2006; Seidl *et al.*, 2006). A glycosylation mechanism keeps these proteins in a monomeric form necessary to elicit the ISR response (Vargas *et al.*, 2008).

Chitin oligosaccharides act as elicitors of defence responses in plants, and the scavenging of such oligomers is fundamental to the lifestyle of fungal pathogens upon colonization of their hosts (de Jonge *et al.*, 2010). As a mechanism for perceiving chitin, plants probably developed chitinases to release the active polymers from the cell walls of invading fungi, thereby triggering defence responses. Thus, the mycotrophic activity of *Trichoderma* chitinases can also release chito oligosaccharides and indirectly contribute to the induction of this defence mechanism.

Certain secondary metabolites produced by *Trichoderma* exert an antimicrobial effect at high doses but act as MAMPs and as auxin-like compounds at low concentrations. At 1 p.p.m., 6-pentyl- α -pyrone, harzianolide and harzianopyridone activate plant defence mechanisms and regulate plant growth in pea, tomato and canola (Vinale *et al.*, 2008), suggesting that plants' defence mechanisms and their developmental responses to *Trichoderma* share common components. Peptaibols are linear peptide antibiotics of 5 to 20 amino acid residues generated by non-ribosomal peptide synthetase activity. Alamethicin, a 20mer peptaibol from *T. viride*, elicits JA and SA biosynthesis in lima bean (Engelberth *et al.*, 2001), whereas 18mer peptaibols from *T. virens* elicit systemic defences in cucumber against the leaf pathogen *Pseudomonas syringae* pv. *lachrymans* (Viterbo *et al.*, 2007). The protection of tobacco plants against tobacco mosaic virus by *Trichoderma* peptaibols was shown to involve multiple defence signalling pathways (Luo *et al.*, 2010).

Defence signalling pathways induced by *Trichoderma*

Trichoderma had received little attention as a potential inducer of plant resistance until the publication of studies

describing that bean root colonization by *T. harzianum* was effective in inducing defence responses (De Meyer *et al.*, 1998) and that penetration of *T. asperellum* in the root system triggered ISR in cucumber seedlings (Yedidia *et al.*, 1999).

As a consequence of *Trichoderma* root colonization and MAMP interaction the proteome and transcriptome of plant leaves are systemically affected (reviewed by Shores *et al.*, 2010). The ISR triggered by *Trichoderma* occurs through the JA/ET signalling pathway similarly to PGPR-ISR (Shores *et al.*, 2005), as confirmed by several authors: (i) cerato-platanin Sm1 is required for *T. virens*-mediated ISR against *Colletotrichum graminicola* in maize (Djonović *et al.*, 2007), (ii) *Trichoderma* treatment of JA/ET-deficient *Arabidopsis* genotypes leads to enhanced susceptibility to *Botrytis cinerea* (Korolev *et al.*, 2008), (iii) ISR triggered by PGPR and *Trichoderma* converges upstream from MYB72, an early key component of the onset of ISR (Segarra *et al.*, 2009). However, other studies have shown that in the *T. asperellum*–cucumber interaction the induction of plant responses is a time- and concentration-dependent phenomenon, and in the first hours of contact a SAR-like response is observed, with an increase in SA and peroxidase activity. In fact, a systemic increase in SA and JA levels was observed after inoculation of high densities of *Trichoderma* (Segarra *et al.*, 2007). Gallou *et al.* (2009) also observed that the defence response of *T. harzianum*-challenged potato to *Rhizoctonia solani* was dependent on JA/ET and SA.

Recent findings include: (i) the colonization of *Arabidopsis* roots by *T. atroviride* induces a delayed and overlapping expression of the defence-related genes of the SA and JA/ET pathways against biotrophic and necrotrophic phytopathogens, both locally and systemically (Salas-Marina *et al.*, 2011); (ii) *Trichoderma* is able to trigger a long-lasting upregulation of SA gene markers in plants unchallenged by pathogens, although when plants are infected by a pathogen such as *B. cinerea*, the pretreatment with *Trichoderma* may modulate the SA-dependent gene expression and, soon after infection, the expression of defence genes induced through the JA signal transduction pathway occurs, causing ISR to increase over time (Tucci *et al.*, 2011); and (iii) colonization of *Arabidopsis* root by *T. asperellum* produces a clear ISR through an SA signalling cascade, and both the SA and JA/ET signalling pathways combine in the ISR triggered by cell-free culture filtrates of *Trichoderma* (Yoshioka *et al.*, 2011).

Plant growth promotion and tolerance to abiotic stress

Certain *Trichoderma* spp. have beneficial effects on plant growth and enhance resistance to both biotic and abiotic stresses. Early work revealed that *Trichoderma* promotes growth responses in radish, pepper, cucumber and tomato (Baker *et al.*, 1984; Chang *et al.*, 1986). Further studies demonstrated that *Trichoderma* also increases root

development and crop yield, the proliferation of secondary roots, and seedling fresh weight and foliar area (Harman, 2000). Moreover, *T. harzianum* can solubilize several plant nutrients (Altomare *et al.*, 1999), and the colonization of cucumber roots by *T. asperellum* has been shown to enhance the availability of P and Fe to plants, with significant increases in dry weight, shoot length and leaf area (Yedidia *et al.*, 2001).

Tucci *et al.* (2011) have recently demonstrated the effects of the plant genetic background on the outcome of the interaction between different tomato lines and two biocontrol strains of *T. atroviride* and *T. harzianum*, and in at least one tomato cultivar the *Trichoderma* treatment did not exert any plant growth promotion effect and was even seen to be detrimental. The growth-promoting activity of *T. atroviride* on tomato seedlings has been suggested to be associated with the reduced ET production resulting from a decrease in its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) through the microbial degradation of indole-3-acetic acid (IAA) in the rhizosphere, and/or through the ACC deaminase (ACCD) activity present in the micro-organism (Gravel *et al.*, 2007). Putative sequences of ACCD have been found in *Trichoderma* genomes (Kubicek *et al.*, 2011), and RNAi silencing of the ACCD gene from *T. asperellum* revealed a decreased ability of the mutants to promote the root elongation of canola seedlings, suggesting a role for ACCD in root growth promotion (Viterbo *et al.*, 2010). Moreover, *Trichoderma* spp. produce auxins that are able to stimulate plant growth and root development (Contreras-Cornejo *et al.*, 2009). As indicated above, an auxin-like effect has been observed in etiolated pea stems treated with harzianolide and 6-pentyl- α -pyrone, the major secondary metabolites produced by different *Trichoderma* strains (Vinale *et al.*, 2008). Maize rhizosphere colonization by *T. virens* also induces higher photosynthetic rates and systemic increases in the uptake of CO₂ in leaves (Vargas *et al.*, 2009).

The beneficial effects of *Trichoderma* on abiotic stress have been well documented (Donoso *et al.*, 2008; Bae *et al.*, 2009), although the mechanisms controlling multiple plant stress factors are still unknown. Recently, Mastouri *et al.* (2010) reported that the treatment of tomato seeds with *T. harzianum* accelerates seed germination, increases seedling vigour and ameliorates water, osmotic, salinity, chilling and heat stresses by inducing physiological protection in plants against oxidative damage. These responses are comparable with the effects induced in plants by *P. indica*, which shows strong growth-promoting activity during its symbiosis with a broad spectrum of plants and induces resistance to fungal diseases and tolerance to salt stress (Vadassery *et al.*, 2009). A common mechanism through which beneficial fungi and PGPR enhance plant tolerance to these abiotic stresses could be the amelioration of damage caused by ROS accumulation in stressed plants (Mastouri *et al.*, 2010).

***Trichoderma*-plant cross-talk model**

Plant immunity and development are interconnected in a network of hormone-signalling pathways (Pieterse *et al.*, 2009). There is a cross-communication between SA, JA and ET, the central players in defence, and the response pathways of other hormones such as abscisic acid, commonly associated with plant development and abiotic stress, IAA, related to plant growth and lateral root development, and gibberellins, which control plant growth by regulating the degradation of growth-repressing DELLA proteins. In *Trichoderma*, the ACCD activity reduces the availability of ACC necessary for ET biosynthesis (Fig. 2). Reductions in ET promote plant growth via gibberellin signalling by increasing the degradation of DELLA proteins. Moreover, gibberellins may control the onset of JA- and SA-dependent defence responses of the plant through the regulation of DELLA protein degradation. ET and IAA in the roots can reciprocally regulate each other's biosynthesis (Stepanova *et al.*, 2007) and, according to this observation, *Trichoderma* IAA contributes to exogenous auxin-stimulated ET biosynthesis via ACC synthase, which in turn triggers an increase in abscisic acid biosynthesis. Depending on the timing and outcome of *Trichoderma* stimuli, phytohormone homeostasis will control plant development and immune responses.

Transgenic plants expressing *Trichoderma* genes

The pioneering work of Lorito *et al.* (1998) demonstrated that *Trichoderma* genes can be expressed functionally in plants to confer beneficial features, mainly in the control of plant diseases. In that work, high expression levels of the *T. harzianum* endochitinase gene *chit42* were obtained in different plant tissues, with no visible effect on plant growth and development. Selected transgenic lines of tobacco and potato were highly tolerant or completely resistant to the leaf pathogens *Alternaria alternata*, *Alternaria solani* and *B. cinerea*, and the soil-borne pathogen *Rhizoctonia solani*. *chit42* expression increased resistance to *Venturia inaequalis*, but reduced plant growth, in apple (Bolar *et al.*, 2000) and significantly increased the resistance of broccoli to attack by *Alternaria brassicicola* (Mora & Earle, 2001). The expression of *chit42* in lemon enhanced resistance to *Phoma tracheiphila* and *B. cinerea*, a significant correlation between resistance and transgene expression being observed, with an upregulation of ROS and JA/ET-responsive genes (Gentile *et al.*, 2007; Distefano *et al.*, 2008). The homologous *chit42* gene from *T. virens* was able to enhance resistance against *R. solani* when it was expressed in rice (Shah *et al.*, 2009). Other *Trichoderma* chitinase genes have been used to generate transgenic plants resistant to fungal diseases: (i) tobacco cell cultures expressing the *T. harzianum* endochitinase *chit40* gene released the enzyme into the medium and were able to inhibit the conidial germination of the post-harvest pathogen *Penicillium digitatum* (Brants & Earle, 2001); (ii) transgenic cotton plants expressing the *T. virens*

osmotic stresses when it was expressed in *Arabidopsis* (Hermosa *et al.*, 2011), and transgenic tobacco plants expressing a *T. virens* glutathione transferase to improve their remediation and xenobiotic degradation potential (Dixit *et al.*, 2011).

Conclusions

Abiotic stress tolerance in plants is accompanied by growth inhibition after overexpression of heat-shock genes of plant origin (Cazalé *et al.*, 2009). Thus, an important biotechnological advantage has been the expression of a *T. harzianum hsp70* gene in *Arabidopsis* to induce resistance to high temperatures, high salinity and drought without loss of vigour and growth or developmental alterations (Montero-Barrientos *et al.*, 2010), probably due to the heterologous nature of the transgene. Recent examples of biotechnological solutions from *Trichoderma* are the *T. harzianum Thkell* gene, encoding a kelch-repeat protein involved in the modulation of glucosidase activity that enhanced seed germination and plant tolerance to salt and

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