

# 1 Mechanisms of Growth Promotion by Members of the Rhizosphere Fungal Genus *Trichoderma*

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## 1.1 Introduction

*Trichoderma* species are cosmopolitan filamentous fungi found in agricultural, native prairie, forest, salt marsh, and desert soils of all biomes (rainforests, savannas, deserts, grasslands, temperate deciduous forest, temperate, conifer forest, Mediterranean scrub, taiga and tundra), as well as in lake water, dead plant material, living roots of virtually any plant species, seeds and air (Atanasova *et al.*, 2013; Mukherjee *et al.*, 2013; Waghund *et al.*, 2016). The ability of *Trichoderma* spp. to thrive in such a wide range of habitats is linked to their capability to produce a number of bioactive molecules, such as lytic enzymes, antibiotics and multiple other secondary metabolites.

Rhizosphere competency is widespread among the *Trichoderma* and many strains are considered opportunistic plant endophytes frequently found in symbiotic relationships with diverse crops (including maize, tomato, cucumber, cotton, cocoa, etc.), ornamental flowers, grasses, palms, ferns, trees, etc. (Harman, 2000; Harman *et al.*, 2004; Sobowale *et al.*, 2007; Hohmann *et al.*, 2011; Keswani *et al.*, 2013; Cripps-Guazzone, 2014; Singh *et al.*, 2016). Successful rhizosphere competence and endophytism are subject to host specificity and changes in abiotic environmental factors (Cripps-Guazzone, 2014).

*Trichoderma* spp. induce plant growth by direct and indirect mechanisms. Direct mechanisms include the facilitation or

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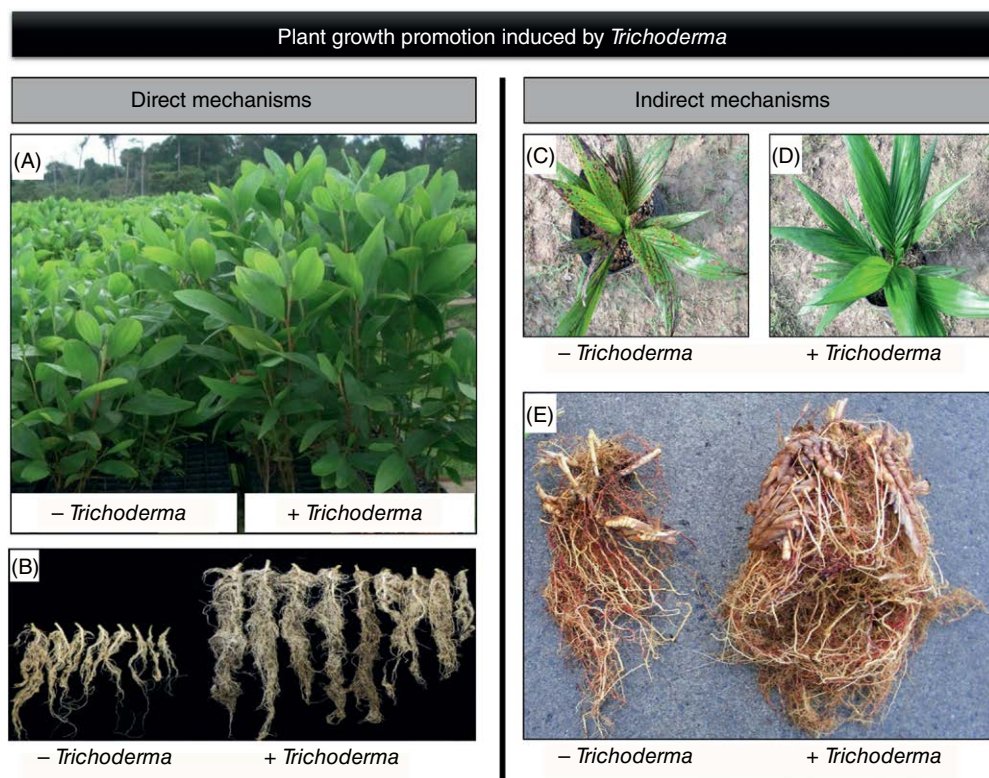
increment of nutrients uptake such as phosphate solubilization, iron sequestration and production of secondary metabolites, including phytohormones and volatile or non-volatile compounds (Vinale *et al.*, 2012; Qi and Zhao, 2013; Saravanakumar *et al.*, 2013; Zhao *et al.*, 2014; Borges Chagas *et al.*, 2015; Lee *et al.*, 2015; Li *et al.*, 2015; Bisen *et al.*, 2016; Garnica-Vergara *et al.*, 2016). Indirect mechanisms include biocontrol activity against plant pathogens (bacteria, fungi and nematodes) and the ability to impart abiotic stress tolerance within the plant (Bruce *et al.*, 1984; Bae *et al.*, 2009; Shukla *et al.*, 2012; Yang *et al.*, 2012; Qi and Zhao, 2013; Vinale *et al.*, 2013; Contreras-Cornejo *et al.*,

2014; Stewart and Hill, 2014; Zhao *et al.*, 2014; Kottb *et al.*, 2015; Shukla *et al.*, 2015; Pandey *et al.*, 2016; Zachow *et al.*, 2016) (Fig. 1.1).

## 1.2 *Trichoderma* Plant Growth Promotion: Direct Mechanisms

### 1.2.1 Nutrient acquisition

Phosphorus and iron are the key elements for plant growth and, although these nutrients are abundant in nature, they are poorly accessible to plants (de Santiago *et al.*, 2013).



**Fig. 1.1.** Plant growth promotion induced by *Trichoderma* spp. (A-B) Direct mechanisms of induction (A). Plant growth promotion induced by *Trichoderma* (+) in *Acacia mangium* in comparison to control plants (-). In this experiment plants were free of any chemical pesticide or fertilizer. (B) Root growth promotion induced by *Trichoderma* in canola. Roots of plants untreated (left) and roots of plants inoculated with a mixture of *T. atroviride* (right). (C-E) Indirect mechanisms of induction (C), effect of leaf spot disease on untreated oil palm seedlings (D) and treated with endophytic *Trichoderma* spp. (E). Silvergrass roots grown in soil infested with *Rhizoctonia solani* (left) and treated plants with commercial product based on a mixture of *Trichoderma* isolates (right).

Microbial communities modify nutrient cycling in the rhizosphere, affecting nutrient availability to plants. *Trichoderma* spp. secrete diverse molecules, including siderophores, organic acid compounds and proteins that contribute to the solubility of inorganic phosphate and iron (Kapri and Tewari, 2010; Khan *et al.*, 2010; de Santiago *et al.*, 2013; Saravanakumar *et al.*, 2013; Borges Chagas *et al.*, 2015).

#### Phosphate solubilization

Phosphorus (P) is the second most limiting nutrient to plant growth behind nitrogen (N) (Condrón, 2004) and, as a result, pasture and crops require the input of organic P through fertilizers (Koning *et al.*, 2008; Simpson *et al.*, 2014; Desmidt *et al.*, 2015) to reach the growth levels needed for sustainable farming. Phosphate fertilizers contain phosphate rock which is mined from natural deposits and is therefore non-renewable (Desmidt *et al.*, 2015). The uptake of organic P by plants is low, somewhere between 5% and 30% depending on the soil alkalinity (Condrón, 2004). The rest forms insoluble inorganic compounds with aluminium (Al), iron (Fe) and calcium (Ca), unavailable for plant uptake (Ward *et al.*, 1996; Heffer and Prud'homme, 2008). Phosphate-solubilizing micro-organisms, such as fungi and bacteria, play a major role in the transformation of insoluble soil P into soluble available forms (Rodríguez and Fraga, 1999; Khan, 2009), and therefore are potential bio-fertilizers. *Trichoderma* spp. have known P solubilizing activity (Kapri and Tewari, 2010; Mukherjee *et al.*, 2013; Borges Chagas *et al.*, 2015). The potential mechanism for phosphate solubilization might be acidification either by proton extrusion or association with ammonium assimilation.

#### Siderophores

Iron (Fe) is one of the most abundant elements on earth, however it is present as ferric ions in the soil which are not very soluble and are consequently inaccessible to plants (Lehner *et al.*, 2013). Siderophores are molecules that solubilize Fe and as a consequence

alter nutrient availability in soil environments for microorganisms and plants (Vinale *et al.*, 2013). Lehner *et al.* (2013) analysed eight different strains of *Trichoderma*, including *T. atroviride* IM206040, *T. asperellum*, *T. gamsii*, *T. hamatum*, *T. virens* Gv29.8, *T. harzianum*, *T. polysporum* and *T. reesei* QM6a and observed that on average *Trichoderma* spp. produced 12 to 14 siderophores, with six common to all species. In *Trichoderma* spp., intracellular siderophores are synthesized by three non-ribosomal protein synthases (NRPs), which are present as a cluster in the genome (Mukherjee *et al.*, 2012a; Zeilinger *et al.*, 2016). The role of the NRP6 from *T. virens* has been related to the biosynthesis of 10 of 12 extracellular secreted siderophores. Harzianic acid is a secreted siderophore molecule synthesized by *T. harzianum* and this molecule has plant growth-promoting and antifungal activity (Vinale *et al.*, 2013). The role of siderophores in aiding competition with other microbes in the rhizosphere or in providing Fe to the plants has not been completely explored and there is still much work to be done to understand the role of these molecules in the plant-microbe-rhizosphere interaction and its relation to plant growth promotion.

#### Synthesis of secondary metabolites

Secondary metabolites produced by plant-associated microbes change the chemical and physical properties of soil, increasing iron, nitrogen or phosphorus availability (Bitas *et al.*, 2013). Moreover, beneficial microorganisms are able to manipulate hormone signalling pathways in the host plant and as a consequence enhance plant growth (Kunkel and Brooks, 2002; Sofo *et al.*, 2011; Spaepen and Vanderleyden, 2011). The chemical composition of secondary metabolites produced by *Trichoderma* is diverse (Vinale *et al.*, 2012; Keswani *et al.*, 2014; Bansal and Mukherjee, 2016; Zeilinger *et al.*, 2016). *Trichoderma* metabolites directly influence plant physiology by modulating hormone activity in the plant, affecting nutrient solubility or by combating plant pathogens (Keswani *et al.*, 2014).

**HORMONES.** *Trichoderma* synthesizes 3-indoleacetic acid (IAA), the major auxin in plants (Yue *et al.*, 2014; Enders and Strader, 2015), acting as a plant growth promoter (Contreras-Cornejo *et al.*, 2009). *T. virens* synthesizes indolic compounds, viz. IAA, indole-3-acetaldehyde (IAAld), indole-3-ethanol (tryptophol) and indole-3-carboxaldehyde (ICAld) (Contreras-Cornejo *et al.*, 2009; 2011). IAA, IAAld and ICAld synthesized by *T. virens* have auxin activity in *A. thaliana*; however tryptophol did not show significant auxin activity in this model plant (Contreras-Cornejo *et al.*, 2009; 2011). The exact mechanism and enzymes involved in the synthesis of IAA are unknown but in *T. virens* it has been suggested that indole-3-ethanol and indole-3-acetaldehyde are key components for the IAA biosynthetic pathway (Contreras-Cornejo *et al.*, 2009). Nevertheless, not all plant growth-promoting *Trichoderma* strains synthesize auxins, at least under laboratory conditions, suggesting that additional mechanisms are involved (Hoyos-Carvajal *et al.*, 2009). Inhibition of ethylene represents the best studied mechanism of plant growth promotion induced by microorganisms (Nascimento *et al.*, 2014). *T. asperellum* produces 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which cleaves ACC, the immediate precursor of the plant hormone ethylene, to produce  $\alpha$ -ketobutyrate and ammonia (Todorovic and Glick, 2008). Inoculation of microorganisms that synthesize ACC deaminase, such as *Trichoderma* spp., induces plant growth promotion by the reduction of ethylene (Viterbo *et al.*, 2010).

**VOLATILE ORGANIC COMPOUNDS (VOCs).** Plant-associated fungi produce a great variety of VOCs which comprise mainly pyrones, terpenes, alcohols, ketones, alkanes and alkenes (Korpi *et al.*, 2009). The biosynthesis of fungal VOCs often coincides with certain developmental stages like spore formation and some of these compounds are produced in association with mycotoxins (Wilkins *et al.*, 2003). VOCs synthesis depends on nutrient availability, pH, temperature and light, and is species/strain-specific (Zeilinger

*et al.*, 2016). Overall, microbial VOCs promote plant growth, increase crop yield and protect host plants against pathogenic organisms.

*Trichoderma* volatiles are able to induce beneficial effects on *A. thaliana* seedlings. VOCs emitted by *T. viride*, *T. atroviride* and *T. virens* cultures in a shared atmosphere with *A. thaliana*, without direct contact, resulted in larger plants, earlier flowering, and enhancement of lateral root development (Hung *et al.*, 2013; Contreras-Cornejo *et al.*, 2014; Salazar-Badillo *et al.*, 2015). However, Kottb *et al.* (2015) reported that after the interaction of *A. thaliana* with VOCs emitted by *T. asperellum* IsmT5, there was an accumulation of anthocyanin pigments, a rise by 47% of the trichome density, an increased level of  $H_2O_2$  as a sign of the activation of plant defence responses, 97% increase in camalexin accumulation, a higher respiration activity (40% more than the control group) and greater concentrations of salicylic acid and abscisic acid. Overall *A. thaliana* plants exposed to *Trichoderma* volatiles showed improved survival strategies and defence responses in these two different experiments.

The emission of biologically active VOCs by *Trichoderma* has been reported frequently since the 1950s. For several years, the plant growth promotion effects and fungal plant-pathogen inhibition were attributed to carbon dioxide, ethanol, acetaldehyde and acetone (Tamimi and Hutchinson, 1975), but improvements in analytical techniques revealed the diversity in volatile profiles of several species of *Trichoderma*. Analysis of VOCs produced by *T. atroviride*, demonstrated the presence of 25 different fungal metabolites including alcohols, ketones, alkenes, furanes, pyrenes, monoterpenes and sesquiterpenes (Stoppacher *et al.*, 2010).

Within the range of metabolites produced by genus *Trichoderma*, 6-pentyl-2H-pyron-2-one (6-PP) is often reported as the major volatile produced by this fungus which promotes plant growth and influences root architecture. *A. thaliana* root response to 6-PP involves components of auxin transport and signalling and the ethylene response modulator EIN2 (Garnica-Vergara *et al.*, 2016).

### 1.3 *Trichoderma* Plant Growth Promotion: Indirect Mechanisms

#### 1.3.1 Biocontrol of plant disease

The potential of *Trichoderma* species as biocontrol agents of plant diseases was first recognized in the early 1930s (Weindling, 1932) and since then *Trichoderma* species have been reported to control many plant diseases of fruit and vegetable crops. For example, Matei and Matei (2008) reported that *T. harzianum* P8 was able to control *Botrytis cinerea* on strawberry cultivars by hyperparasitism. Perello *et al.* (2009) used *T. harzianum* and *T. koningii* to protect wheat from leaf blotching caused by *Septoria tritici* in Argentina. The same authors also used these species to control tan spot, caused by *Pyrenophora tritici-repentis* on wheat (Perello *et al.*, 2008). Harman (2000) reported that *T. harzianum* T22 controlled diseases caused by *Fusarium* species on tomatoes. *Trichoderma* has also been used in wood preservation, with Vanneste *et al.* (2002) reporting that *T. harzianum* could provide better control of sapstain than the standard fungicide on *Pinus radiata*. These studies and others have culminated in the development of commercial products/prototype formulations of several *Trichoderma* species for the protection of a number of crops in the USA, India, Israel, New Zealand and Sweden (Howell, 2003; Kandula *et al.*, 2015).

*Trichoderma* achieves successful biocontrol through a multitude of mechanisms including induced systemic resistance, mycoparasitism, antibiosis, microbial competition and direct growth promotion. Direct growth promotion results in stronger and healthier plants, which, in turn, are better able to cope with disease and abiotic stresses (Bisen *et al.*, 2015; Mishra *et al.*, 2015). Biocontrol itself in turn promotes plant growth indirectly by protecting the plant from pathogens and subsequent yield/growth loss.

#### *Induced systemic resistance*

There are three recognized pathways of induced resistance in plants (Harman *et al.*, 2004): the salicylic acid pathway, the jasmonic

acid pathway and the non-pathogenic root-associated bacteria induced pathway. In the salicylic and jasmonic acid pathways the production of pathogenesis-related proteins (PR) (antifungal chitinases, glucanases, thaumatinins, and oxidative enzymes) are triggered by the attack of pathogenic microorganisms and the wounding or necrosis-inducing plant pathogens (herbivory by insects). In the non-pathogenic root-associated bacteria induced pathway, the PR proteins are not induced by root colonization in the absence of attack by plant-pathogenic microorganisms.

De Meyer *et al.* (1998) were the first to demonstrate that *Trichoderma* spp. could induce resistance in plants. They reported that bean plants grown in soil treated with *T. harzianum* T39 showed fewer disease symptoms after *B. cinerea* inoculation to the leaves compared with untreated control plants, even though T39 was only present on the roots and not on the foliage. Yedidia *et al.* (2003) presented conclusive evidence for the induction of a systemic response against angular leaf spot of cucumber caused by *Pseudomonas syringae* pv. *lachrymans* following application of *T. asperellum* to the root system. Disease symptoms were reduced by as much as 80%, corresponding to a reduction of two orders of magnitude in numbers of bacterial cells in leaves of plants pre-treated with *T. asperellum*. Similar studies have now been carried out with a wide range of plants, including both monocotyledons and dicotyledons and with multiple *Trichoderma* species and strains (Harman *et al.*, 2004), and these have demonstrated that induced resistance can be mediated by *Trichoderma* spp.

#### *Mycoparasitism*

A key characteristic of members of the genus *Trichoderma* is their ability to parasitize other fungi, some of which are plant pathogens, and many instances of successful biocontrol with *Trichoderma* species have been ascribed to this mechanism. Mycoparasitism occurs in several steps, first, *Trichoderma* spp. detect other fungi and grow tropically towards them (Chet *et al.*, 1981). Uncharacterised diffusible factors act as elicitors of proteases (*prb1*) which are

directly associated with the mycoparasitic activity of *T. atroviride* (Geremia *et al.*, 1993; Cortes *et al.*, 1998; Olmedo-Monfil *et al.*, 2002; Steyaert *et al.*, 2004). Brunner *et al.* (2003) suggested that diffusion of low levels of an extracellular exochitinase catalyses the release of cell-wall oligomers from target fungi, and this in turn induces the expression of fungitoxic endochitinases which also diffuse and begin the attack on the target fungus before contact is actually made (Viterbo *et al.*, 2002). Once in direct contact, *Trichoderma* produces several fungitoxic cell wall degrading enzymes (CWDEs) (Chet *et al.*, 1998; Steyaert *et al.*, 2003; Lorito *et al.*, 2010). These enzymes function by breaking down the polysaccharides, chitin, and  $\beta$ -glucans which form fungal walls, thereby destroying the cell wall integrity of pathogenic fungi (Howell, 2003). Signalling genes/pathways involved in mycoparasitism include the kinase Tvk1/TmkA from *T. virens* and Tmk1 from *T. atroviride*, which are negative regulators of hydrolytic enzymes and antibiotics. The corresponding gene deletion mutants were more effective in controlling plant disease caused by *R. solani* than the commercial chemical fungicides in beans (Reithner *et al.*, 2007; Mukherjee *et al.*, 2013).

Howell (1982) observed *T. virens* (formerly *Gliocladium virens*) parasitizing *R. solani* by coiling around and penetrating the hyphae. In *T. atroviride* IMI206040, Tga1 and Tga3, two G-protein  $\alpha$  subunits from the cAMP signalling pathway, regulate coiling (Rocha-Ramirez *et al.*, 2002). In addition, Tga1 regulates the production of lytic enzymes and biosynthesis of antifungal metabolites that impact mycoparasitism (Reithner *et al.*, 2005) while Tga3 regulates secretion of CWDEs but not their biosynthesis (Zeilinger *et al.*, 2016). Recent comparative analysis of the genome, secretome and transcriptome of the three species: *T. atroviride* IMI206040, *T. virens* Gv29.8, and *T. reesei* QM6a, indicated mycoparasitism as the ancestral lifestyle of *Trichoderma* (Kubicek *et al.*, 2011; Atanasova *et al.*, 2013).

### Antibiosis

Antibiosis is the process of secretion of anti-microbial compounds by antagonistic microbes to suppress and/or kill pathogenic

microbes in the vicinity of their growing area (Schirmbock *et al.*, 1994). *Trichoderma* produces many secondary metabolites with antibiotic activities and their production is species/strain dependent (Mukherjee *et al.*, 2012b; Zeilinger *et al.*, 2016). Ghisalberti and Sivasithamparam (1991) classified the secondary metabolites into three categories: (i) volatile antibiotics, eg. 6-pentyl- $\alpha$ -pyrone (6-PP) and most of the isocyanide derivatives; (ii) water-soluble compounds, i.e. heptelidic acid or koniginic acid; and (iii) peptaibols, which are linear oligopeptides of 12–22 amino acids rich in  $\alpha$ -aminoisobutyric, N-acetylated at the N-terminus and containing an amino alcohol at the C-terminus. Lorito *et al.* (1996) investigated the activity of peptaibols and cell wall hydrolytic enzymes produced by *T. harzianum* in the antagonism of *B. cinerea*. Peptaibols trichorzianin TA and TB inhibited  $\beta$ -glucan synthase activity in the host fungus. The inhibition was synergistic with *T. harzianum*  $\beta$ -1, 3-glucanase and prevented the reconstruction of the pathogen cell wall, which facilitated the action of the glucanase and enhanced the fungicidal activity. Antibiotics probably act synergistically with lytic enzymes.

### Competition

Competition for carbon, nitrogen and other growth factors, together with competition for space or specific infection sites, is an indirect mechanism by which *Trichoderma* controls plant pathogens (Vinale *et al.*, 2008). Gullino (1992) reported that *T. harzianum* was able to control *B. cinerea* on grapes by colonizing blossom tissue and excluding the pathogen from infection sites. Competition for nutrients is the major mechanism used by *T. harzianum* to control *Fusarium oxysporum* f. sp. *melonis* (Sivan and Chet, 1989). Benitez *et al.* (2004) showed that *Trichoderma* has a strong capacity to mobilize and take up soil nutrients which make it more efficient and competitive than other soil microbes.

### 1.3.2 Abiotic Stress Tolerance

There is increasing evidence to show that *Trichoderma* can protect plants from the

adverse effects of abiotic stress. Stress tolerance in turn results in promotion of growth. Drought tolerance induced by *Trichoderma* have been observed in multiple host plants, including rice, maize, cocoa, wheat and *A. thaliana* (Bae *et al.*, 2009; Shukla *et al.*, 2012; Zaidi *et al.*, 2014; Contreras-Cornejo *et al.*, 2015; Shukla *et al.*, 2015; Chandra and Gaur (2016); Pandey *et al.*, 2016; Rawat *et al.*, 2016). Drought tolerance by *Trichoderma* appears to be strain-specific (Shukla *et al.*, 2012; Rawat *et al.*, 2016). *Trichoderma virens* and *T. atroviride* synthesize abscisic acid (ABA) that modulate stomatal aperture closure and consequently protection against loss of water (Contreras-Cornejo *et al.*, 2015). In wheat, maize and rice, H<sub>2</sub>O<sub>2</sub> content significantly increases in response to drought, however inoculation with *Trichoderma* spp. can significantly reduce the H<sub>2</sub>O<sub>2</sub> content as compared with the control plants (Shoresh and Harman, 2008; Rawat *et al.*, 2012; Rawat *et al.*, 2016). During the plant–*Trichoderma* interaction, *Trichoderma* induces an increased synthesis of antioxidative enzymes in the host plants, these include superoxide dismutases (SOD), peroxidases, glutathione-reductases and glutathione-S-transferases (GST), as well as other detoxifying enzymes in leaves (Shoresh and Harman, 2008).

Another strategy used by *Trichoderma* to provide stress tolerance to its host plant is via the ethylene pathway, where *Trichoderma* mutants unable to synthesise ACC deaminase are less effective in providing tolerance to salt stress, suggesting that *Trichoderma*, similarly to ACC deaminase-producing bacteria, can ameliorate plant growth under conditions of abiotic stress, by lowering detrimental increases in ethylene levels (Brotman *et al.*, 2013).

## 1.4 The ‘Omics’ of *Trichoderma*

Despite the importance of *Trichoderma* only seven species of *Trichoderma* corresponding to 10 strains have been fully sequenced and are publicly available (<http://genome.jgi-psf.org/>) (Table 1.1).

Some members of the genus *Trichoderma* such as *T. virens*, *T. harzianum*, *T. atroviride*, *T. hamatum*, *T. asperellum* and *T. ovalisporum* have the capacity to colonise roots and develop a close interaction with their host plant (Bailey *et al.*, 2006; Alfano *et al.*, 2007; Shoresh and Harman, 2008; Moran-Diez *et al.*, 2015); however, the exact mechanisms that regulate these symbiotic interactions are not fully characterised. More recently, high-dimensional biology, transcriptomics and proteomics have been used to unravel the regulatory mechanisms of *Trichoderma* spp. as plant symbionts (Bailey *et al.*, 2006; Marra *et al.*, 2006; Alfano *et al.*, 2007; Chacon *et al.*, 2007; Segarra *et al.*, 2007; Shoresh and Harman, 2008; Samolski *et al.*, 2009; Mehrabi-Koushki *et al.*, 2012; Lamdan *et al.*, 2015; Moran-Diez *et al.*, 2015; Schmoll *et al.*, 2016). However, much is still unknown and further high-throughput omics technologies are essential to understanding the complexity of biological processes that drive *Trichoderma*–plant interactions and plant growth promotion.

### 1.4.1 *Trichoderma*–plant interaction transcriptomics

In general, it has been observed that root colonization by *Trichoderma* causes transcriptional changes in genes involved in metabolism and stress resistance in both the plant and the fungus (Bailey *et al.*, 2006; Samolski *et al.*, 2009; Shoresh *et al.*, 2010; Hermosa *et al.*, 2012; Brotman *et al.*, 2013), which in turn promotes growth of the host plant. For example, a macroarray study showed that cacao gene expression profiles in response to endophytic association with four different growth-promoting strains of *Trichoderma* were highly similar (Bailey *et al.*, 2006). The majority of up-regulated plant genes were related to environmental stress response. In contrast, the *Trichoderma* expressed genes were mainly involved in nutrient acquisition and cell functionality (Bailey *et al.*, 2006). Using the plant model *A. thaliana*, Brotman *et al.* (2013)



**Table 1.1.** Publicly available *Trichoderma* genomes.

	Genome Assembly size (Mbp)	No. gene models <sup>a</sup>	Read coverage depth	Genome source
<i>Trichoderma asperellum</i> CBS 433.97	40.87	13932	100X	<a href="http://genome.jgi.doe.gov/Trias1/Trias1.home.html">http://genome.jgi.doe.gov/Trias1/Trias1.home.html</a>
<i>Trichoderma asperellum</i> TR356	35.39	12320	120X	<a href="http://genome.jgi.doe.gov/Triasp1/Triasp1.home.html">http://genome.jgi.doe.gov/Triasp1/Triasp1.home.html</a>
<i>Trichoderma atroviride</i> IMI206040	36.1	11863	~8.26X	<a href="http://genome.jgi.doe.gov/Triat2/Triat2.home.html">http://genome.jgi.doe.gov/Triat2/Triat2.home.html</a>
<i>Trichoderma citrinoviride</i> TUCIM 6016	33.22	9737	63.1X	<a href="http://genome.jgi.doe.gov/Trici4/Trici4.home.html">http://genome.jgi.doe.gov/Trici4/Trici4.home.html</a>
<i>Trichoderma harzianum</i> CBS 226.95	40.98	14095	120X	<a href="http://genome.jgi.doe.gov/Triha1/Triha1.home.html">http://genome.jgi.doe.gov/Triha1/Triha1.home.html</a>
<i>Trichoderma harzianum</i> TR274	40.87	13932	100X	<a href="http://genome.jgi.doe.gov/Trihar1/Trihar1.home.html">http://genome.jgi.doe.gov/Trihar1/Trihar1.home.html</a>
<i>Trichoderma longibrachiatum</i> ATCC 18648	40.87	13932	100X	<a href="http://genome.jgi.doe.gov/Trilo3/Trilo3.home.html">http://genome.jgi.doe.gov/Trilo3/Trilo3.home.html</a>
<i>Trichoderma reesei</i> RUT C-30	32.69	9852	47.6X	<a href="http://genome.jgi.doe.gov/TrireUTC30_1/TrireUTC30_1.home.html">http://genome.jgi.doe.gov/TrireUTC30_1/TrireUTC30_1.home.html</a>
<i>Trichoderma reesei</i> QM6a	34.1	9129		<a href="http://genome.jgi.doe.gov/Trire2/Trire2.home.html">http://genome.jgi.doe.gov/Trire2/Trire2.home.html</a>
<i>Trichoderma virens</i> Gv29-8	39	12427	~8.05X	<a href="http://genome.jgi.doe.gov/TriviGv29_8_2/TriviGv29_8_2.home.html">http://genome.jgi.doe.gov/TriviGv29_8_2/TriviGv29_8_2.home.html</a>

<sup>a</sup>Predicted and annotated using the JGI annotation pipeline

observed that *Trichoderma* stimulated plant growth and resistance to saline stress and significantly improved seed germination. Analysis of the up-regulated plant genes show that they were related mainly to osmo-protection and general stress response. Other authors observed the regulation of the WRKY transcription factors and the ADC genes are related with several important biological functions (Sáenz-Mata *et al.*, 2014; Salazar-Badillo *et al.*, 2015). In addition, it has been suggested that similarly to ACC deaminase-producing bacteria, *Trichoderma* can enhance plant growth under conditions of abiotic stress, by lowering the levels of ethylene as well as promoting an increment in antioxidative activity and by the modulation of polyamine content (Brotman *et al.*, 2013; Salazar-Badillo *et al.*, 2015).

#### 1.4.2 Proteomics

To understand the changes occurring in the plant in response to interacting with *Trichoderma*, several studies have been carried out to identify proteome and secretome profiles using gel-based 2-DE gel analysis coupled with LC-MS/MS or MALDI-TOF MS and gel free-based proteomics coupled with LC-MS/MS (Marra *et al.*, 2006; Segarra *et al.*, 2007; Shores and Harman, 2008; Lamdan *et al.*, 2015). Shores and Harman (2008) demonstrated that colonization of maize roots by *T. harzianum* altered the shoot proteome in terms of carbohydrate metabolism, photosynthesis and stress, and this up-regulation may correspond to the enhanced growth promotion response and induce systemic resistance.



Induced systemic resistance is an indirect plant growth promotion mechanism that *Trichoderma* confer to plants. The presence of *T. asperellum* in cucumber roots triggers the salicylic acid and jasmonate pathways in the plant, and increase peroxidases activity, hence conferring protection to cucumber plants against foliar pathogens (Segarra *et al.*, 2007). Remarkably, *T. harzianum* did not alter plant secondary metabolism and protein biosynthesis compared to *T. asperellum*, suggesting that *Trichoderma* spp. may have different strategies to induce plant immune changes. Currently diverse reports indicate that *Trichoderma* induce systemic resistance by releasing not only proteins, but also secondary metabolites (Reithner *et al.*, 2007; Luo *et al.*, 2010; Mukherjee *et al.*, 2012b; Cai *et al.*, 2013; Martinez-Medina *et al.*, 2013; Harel *et al.*, 2014; Sáenz-Mata *et al.*, 2014; Lamdan *et al.*, 2015; Salas-Marina *et al.*, 2015; Salazar-Badillo *et al.*, 2015; Keswani *et al.*, 2016). Overall, the current proteomic studies from *Trichoderma*–plant interaction give us insight of how *Trichoderma* induces changes

in plant metabolism that leads to enhanced growth and immunity to plant pathogens.

## 1.5 Conclusion

*Trichoderma* spp. are best known for their biocontrol capabilities against a range of phytopathogenic microorganisms and increased plant drought tolerance. However, all the attributes of *Trichoderma* are also related to their ability to induce plant growth promotion by direct or indirect mechanisms. The activation of these mechanisms might be dependent on the ability of *Trichoderma* to respond to the environmental conditions and host plant.

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