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Review

Trichoderma as biostimulant: exploiting the multilevel properties of a plant beneficial fungus

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ABSTRACT

Plant biostimulants are formulated with diverse microorganisms and/or substances that are applied to crops with the aim of enhancing growth, development and adaptation to abiotic stress. *Trichoderma*-based products have been particularly successful because of their capacity to control phytopathogenic fungi. Some *Trichoderma* strains have a predominant biostimulant action that makes them unique for their extended use in horticulture. They are safe for humans, livestock and crop plants and in their natural environment colonize plant roots without apparent adverse reactions. Both solid and liquid formulations containing conidia can be used to produce suitable quantities of active and viable inocula during product formulation and field use. The mechanism of phytostimulation by *Trichoderma* involves multilevel communication with root and shoot systems, as it releases into the rhizosphere auxins, small peptides, volatiles and other active metabolites, which promote root branching and nutrient uptake capacity, thereby boosting plant growth and yield. Recent proteomic and genetic data suggest that *Trichoderma* activates the mitogen activated protein kinase 6, transcription factors and DNA processing proteins, which represent promising targets toward formulation of more efficient products.

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1. Introduction

Currently, there are almost 7 billion people in the world for which plants should provide grains, fruits, seeds and energy resources. Moreover, it has been estimated that in the next 50 years total population will increase by at least 1.5 billion people, making it necessary to duplicate crop production in the same time to ensure food provision (IFA, 2012). The so called "green revolution" that employed rice, corn and wheat varieties almost doubled yields from 1.9 tons per hectare in 1950–64 to 3.5 tons in 1985–98. This has resulted in an increase in grain production from 824 million tons in 1960 to almost 2400 million tons in 2011. However, this was possible only thanks to intensive application of nitrogen (N) and phosphate (P) based fertilizers and depletion of soil and water resources (Den Herder et al., 2010). In the coming years, a global P crisis is expected to occur, due to exhaustion of most P reserves. In 2008, the phosphate rock price temporarily spiked at US $500 per ton, more than five times the average price in 2007 (Gilbert, 2009). To farmers, increasing P use efficiency through better agricultural practices is an urgent need to save money while maintaining yields.

Other major challenges of agriculture are globalization and climate change, which are predicted to modify the scale and frequency of emerging plant diseases, and threatening global food security. New highly virulent fungal strains are emerging, which adapt to warmer temperatures and overcome many of the main plant defense genes (Hubbard et al., 2015). Several below-ground characteristics of plants such as root architecture, P uptake and N fixation are promising features to strengthen the innate immune system of plants. Indeed, a more sustainable agriculture requires yield increases and product quality, while reducing the negative impact of agrochemicals on the environment, all of which might be fostered by microbial based biostimulants (Berg, 2009; Xiang et al., 2012; Calvo et al., 2014; Owen et al., 2015).

Microbial based biostimulants have been important since the beginning of agriculture (*i.e. Rhizobium* in legumes) and current expectations include their commercialization as a complement to crop nutrition. The beneficial effects of microbes to plants depend upon sophisticated nutritional and chemical signaling as well as soil and climate factors. Plant roots release sugars, organic acids, amino acids and phenolics, which affect the composition of rhizosphere communities, leading to beneficial relationships (Ortíz-Castro et al., 2009). Symbiosis takes place between crops and soil microoganisms, including plant growth promoting rhizobacteria (PGPR) and plant growth promoting fungi (PGPF), which are considered natural biostimulants. *Trichoderma* spp. belong to a class of PGPF that has been successfully used on a commercial scale for biological control of phytopathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Armillaria mellea* and *Chondrostereum purpureum* (Benítez et al., 2004; El-Komy et al., 2015). There is a great variability on antagonistic capacity and biostimulant action of *Trichoderma* resulting in strains that have a predominant biostimulant action and others that have a predominant antagonistic activity. Therefore, some *Trichoderma* strains are more suitable for biological control as biopesticides and other for stimulating crop growth and nutrient uptake acting as biostimulants (Benítez et al., 2004; Shoresh et al., 2010; Contreras-Cornejo et al., 2013, 2014a; Zhao et al., 2014; Colla et al., 2015a,b).

Trichoderma spp. help plants better resist environmental stresses such as salinity and drought *via* reinforcing plant growth and reprograming gene expression in roots and shoots (Fig. 1). The fungal mycelium secretes different compounds that increase the branching capacity of the root system, thus improving nutrient and water acquisition. This review focuses on recent advances in our understanding of the phytostimulant properties of *Trichoderma*, the underlying signaling mechanisms and their potential applications.

2. Growth and habitat of *Trichoderma*

Trichoderma (teleomorph *Hypocrea*, Ascomycota, Dikarya) is a well-studied fungal genus that currently consists of more than 200 molecularly defined species (Atanasova et al., 2013). *Trichoderma* species are in general considered cosmopolitan and prevalent components of different ecosystems in a wide range of climatic zones (Kubicek et al., 2008). However, some species are ubiquitous while others are limited to specific geographical areas (Harman et al., 2004). Members of this genus are frequently found parasitizing other fungi, on dead wood and bark, in soil and rhizosphere, in marine sponges (Paz et al., 2010; Gal-Hemed et al., 2011), associated to woody and herbaceous plants (Jaklitsch, 2009; Chaverri et al., 2011; López-Quintero et al., 2013) and as endophytes (Zhang et al., 2007; Hanada et al., 2008; Mulaw et al., 2010), which exemplifies their ability to occupy various ecological niches. *Trichoderma* species grow and ramify as typical fungal hyphae, 5–10 μm in diameter (Fig. 2A). Most laboratory strains produce asexual spores such as conidia and chlamydospores (Fig. 2B), and in their natural environment some form ascospores in perithecia. Spores have either dispersal or resting functions and are also used as inocula. *Trichoderma* conidiophores appear as paired branches that assume a pyramidal aspect, ending in one or a few phialides. Phialides may be held in whorls or may be penicillate, and can be densely clustered on a wide main axis or solitary. Conidia are pigmented ranging from deep green to nearly grey and are less than 5 μm long and wide (Samuels, 1996). Although chlamydospores were discovered long time ago, our understanding of the genetic basis for their formation and proliferation is scarce. However, chlamydospores are abundantly produced in submerged culture and are the active propagules in some *Trichoderma*-based commercial formulations.

The versatile life style of *Trichoderma* is based on three major nutritional modes: saprotrophy, mycotrophy, and dependence upon plant-derived sugars. Most *Trichoderma* species can live as parasites of other fungi. In fact, *Trichoderma* fruiting bodies have been found on basidiomycetes, and several species of the genus can grow on a wide variety of fungi as necrotrophic hyperparasites (Druzhinina et al., 2011). However, parasitic interactions of a few species have been observed with other organisms and occasionally in immunocompromised humans (Kredics et al., 2003; Casas-Flores and Herrera-Estrella, 2007; Druzhinina et al., 2008). Some species, such as *Trichoderma reesei, Trichoderma subeffusum, Trichoderma luteffusum, Trichoderma polysporum, Trichoderma phellinicola, Trichoderma sulphurea* and *Trichoderma longibranchiatum* are known for their capacity to produce cellulases, and to depend upon degrading dead or decayed organic matter and wood, suggesting that these enzymes are integral to *Trichoderma* nutrition (Jaklitsch, 2011; Xie et al., 2014). An analysis of the *Trichoderma* genomes sequenced up to date, indicates that the capacity to degrade cellulose is most likely present in the whole genus, although probably with varying efficiency. Based on these observations, it has been hypothesized that the *Trichoderma* ancestral species first parasitized on wood-rotting fungi and later explored wood as an alternative ecological niche, switching to living on pre-degraded wood rather than on a fungal host (Rossmann et al., 1999; Druzhinina et al., 2011).

Another niche occupied by *Trichoderma* species is the rhizosphere, which attracts them due to the presence of root-derived sugar and exudates. These strains are called 'rhizosphere competent strains' and they are particularly suitable for use as inoculants due to their long persistence on the roots and beneficial properties (Fig. 2C and D; Vargas et al., 2009; Druzhinina et al., 2011). A mutualistic relationship with plants, in which the fungus penetrates the external tissues of the root system results in several beneficial effects to the plant, while *Trichoderma* benefits from a nutrient rich environment. Numerous *Trichoderma* species have been collected from different crop fields and plant hosts in diverse

climatic zones of all continents (Jaklitsch, 2009; Chaverri et al., 2011; López-Quintero et al., 2013).

3. Uses of *Trichoderma* in horticulture

3.1. Trichoderma *inocula*

The ability of *Trichoderma* to sense, invade, and destroy other fungi has been the major trait behind their commercial success as biopesticides (Verma et al., 2007). Currently, more than 60% of all registered biopesticides contain a single *Trichoderma* isolate, or mixtures of *Trichoderma* species, in principle for greater bioactivity (Fig. 3). The *Trichoderma* species more frequently used in biocontrol and best studied regarding their mechanisms of action are *T. asperellum*, *T. atroviride*, *T. harzianum*, *T. virens*, and *T. viride*, most of which also exhibit high biostimulant action on horticultural crops. For instance, the mixture of three *Trichoderma* species (*T. harzianum*, *T. viride* and *T. virens*) increased plant height, total biomass and improved shoot and root P and N uptake in chickpea in glasshouse and field trials (Rudresh et al., 2005a). Mixtures like the one described in this example may provide a more consistent level of growth promotion and a broader spectrum of activity, due to the variations in the mechanism of action of the different species used, resulting in better performance and commercial success. Nevertheless, the use of different species in a formulation must be carefully examined, since at least *in vitro* different species of *Trichoderma* may antagonize each other (Gómez et al., 1997).

When grown at the rhizosphere or on the root surface, *Trichoderma* is expected to face frequent interactions with other plant microorganisms such as arbuscular mycorrhizal (AM) fungi. Indeed, such interactions have been investigated in the past, with contrasting results. In some cases, inoculation with both fungi either resulted in positive synergistic effects on plants or in the inhibition of plant growth (Rousseau et al., 1996; Chandanie et al., 2009; Colla et al., 2015a,b). Nevertheless, De Jaeger et al. (2011) showed that *Trichoderma harzianum* was able to parasitize the mycelium of an AM fungus, *Glomus* sp., thus affecting its viability. When ^{33}P was measured in the plant and in the fungal mycelium in the presence/absence of *T. harzianum*, an increased uptake of ^{33}P by the AM fungus in the presence of *T. harzianum* was evidenced, possibly related to a stress reaction caused by mycoparasitism. Regarding potential negative effects of *Trichoderma* on AM fungi, Rousseau et al. (1996) showed that *T. harzianum* proliferated at the spore surface and penetrated the thick host wall of *Rhizophagus intraradices* through local hydrolysis of the wall polymers. At an advanced stage of the colonization process, the hyphae of *R. intraradices* were perforated in many places. Using *in vivo* imaging of green fluorescent protein-tagged lines, Lace et al. (2015) investigated the cellular interactions occurring between *Trichoderma atroviride*, *Medicago truncatula* and two *Gigaspora* species

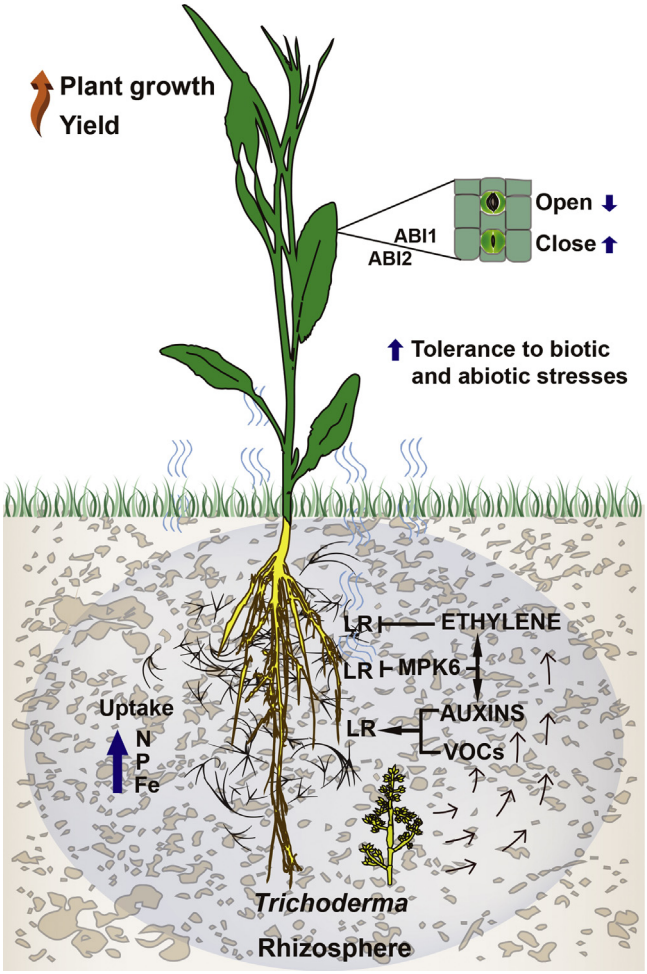


Fig. 1. Biostimulant properties of *Trichoderma* depend on fungal-root communication *via* volatiles, ethylene and auxins. *Trichoderma* metabolites, including volatile blends, and the classic phytohormones indole-3-acetic acid, (IAA) and ethylene, regulate several aspects of root development including lateral root (LR) formation. *T. atroviride* induces the activation of plant mitogen activated protein 6 (MPK6) that translates fungal signals into root cellular responses. Root sensing of *Trichoderma* and its metabolites may increase nutrient uptake and stress tolerance while boosting plant health and yield.

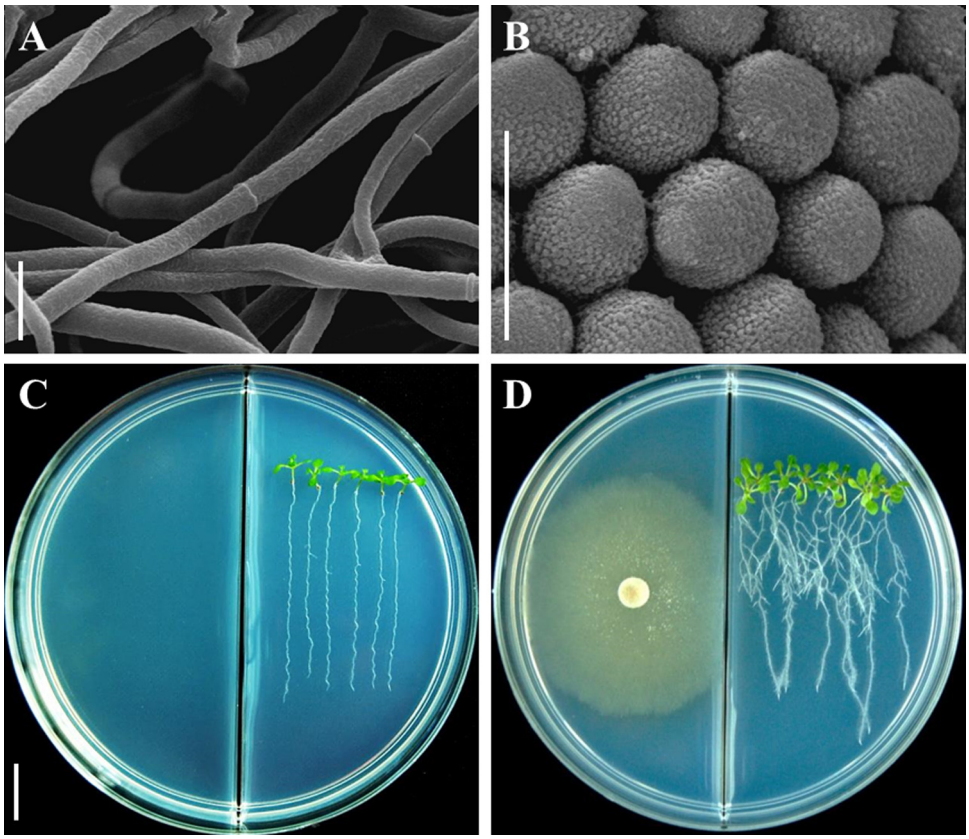


Fig. 2. *Trichoderma virens* propagules and its interaction with *Arabidopsis*. Scanning electron microscopy images of hyphae and conidia of *T. virens*. (A) Hyphae (scale bar 5 μm). (B) Conidia (scale bar 1 μm). (C) 12-day-old *Arabidopsis* (Col-0) seedlings grown on the surface of a divided agar plate containing 0.2X MS medium. (D) 12-day-old *Arabidopsis* exposed to volatiles of *T. virens*. Seedlings were treated with sterilized deionized water at day 7 after germination (left plate), or inoculated with *T. virens* Tv29.8 at the opposite side of the plate (right plate) and photographed 5-days later. Scale bar 1 cm.

under *in vitro* culture conditions. *T. atroviride* parasitized *Gigaspora gigantea* and *Gigaspora margarita*. Despite these data suggest antagonistic *Trichoderma*-AM fungi interactions, recent research indicates that at least some *Trichoderma* combinations with other microbes can improve plant growth (Colla et al., 2015a). The highest shoot, root dry weight, and chlorophyll content in lettuce, tomato and zucchini was observed in a *Glomus–Trichoderma* combination, followed by a single inoculation of *Glomus* or *Trichoderma*, whereas the lowest growth promoting values were recorded in the uninoculated plants. Mixtures of *Trichoderma* and rhizospheric bacteria have also been evaluated for their growth promoting activity. A combination of *T. atroviride* and *Bacillus subtilis* greatly promoted growth of beans in terms of plant dry biomass (43% over the control) compared with 2% for *Trichoderma* alone and 34% for *B. subtilis* alone (Yobo et al., 2011). Similarly, Jisha and Alagawadi (1996) showed that a mixture of *Bacillus polymyxa* and *T. harzianum* enhanced growth of sorghum more than either organism separately. The mechanism of growth promotion by mixtures was attributed to an increased nutrient uptake (Yobo et al., 2009), siderophore production and production of plant growth promoting substances (Yobo et al., 2011). In another set of experiments, a combination of *Rhizobium*, *Bacillus* and *Trichoderma* was applied to chickpea, resulting in greater effects of the mix than any of the individual organisms with increases in germination, nutrient uptake, height, nodulation and total biomass of plants (Rudresh et al., 2005b).

3.2. Mass propagation, formulation and delivery of inoculum

The development of bio-inoculants depends on three crucial components: (1) effectiveness of the strain, (2) production of a high number of effective and viable propagules, and (3) efficient delivery systems that provide a competitive advantage (Harman, 1991). Once an effective bio-inoculant has been identified, the method of mass production, formulation and application should be taken into consideration to stabilize the product during storage and to facilitate its delivery to the plant. Both solid and liquid formulations are used to produce suitable quantities of active and viable inocula of *Trichoderma* mainly as spores (conidia, Fig. 2B), which are more tolerant to adverse environmental conditions during product formulation and field use, in contrast to their mycelial and chlamydospore forms (Amsellem et al., 1999; Howell, 2003; Jin et al., 1991; Papavizas, 1985). Nevertheless, the presence of a mycelial mass is also a key component for the production of relevant metabolites (Benhamou and Chet, 1993). Other ideal properties of a bio-inoculant summarized by Harman et al. (1991) include the following: (1) cost-effective production, (2) preservation against microbial contamination in dry powder form and low water content, and (3) long shelf life. Engineers prefer submerged fermentation systems for mass propagation of *Trichoderma* sp., since they allow better control of nutrients, pH, temperature and other environmental factors thus reducing contamination, while improving biomass production and quality of *Trichoderma* propagules (Whipps, 1997). Consequently, liquid formulation is the preferred approach for biomass production in Europe and North America (Churchill, 1982). Inexpensive growth media such as molasses and yeast extracts are used for liquid formulations (Papavizas et al., 1984). In general terms liquid formulations have limited shelf life and require storage and transportation at low temperatures to sustain viability of the microorganism, resulting in higher costs, unless subjected to a drying process. An alternative

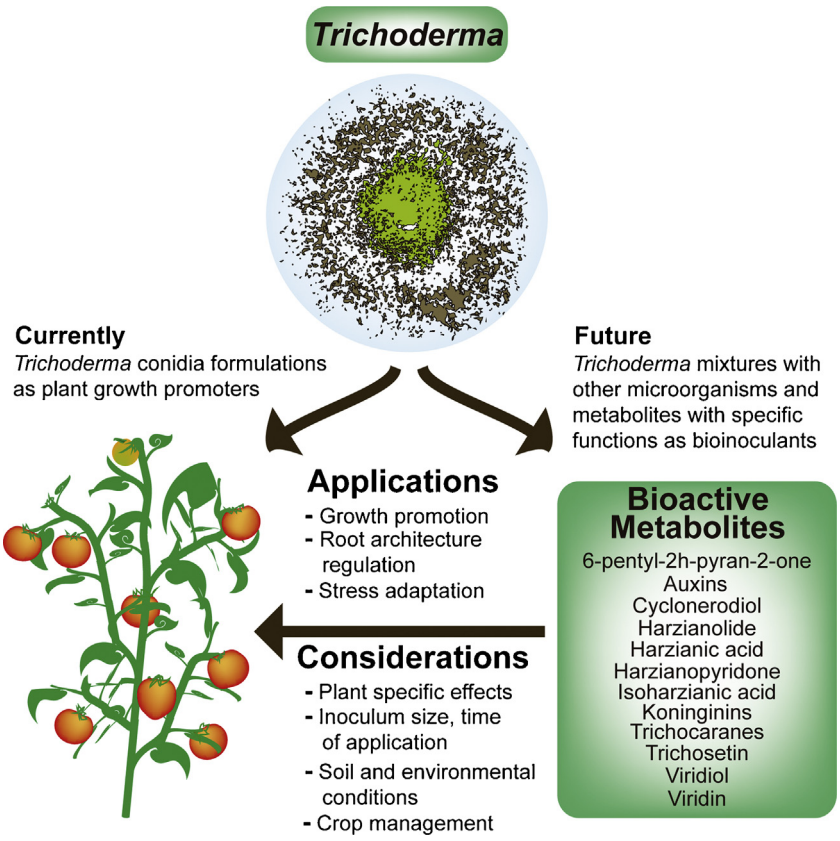


Fig. 3. *Trichoderma* bioinoculants contain conidia for their extended agricultural use. Novel formulations are expected to improve the biostimulant action as novel fungal metabolites with specific functions and plant targets are discovered. Volatiles such as 6-pentyl-2h-pyran-2-one and auxins increase root branching, secondary metabolites and small peptides can improve innate immunity of plants.

for liquid formulations that is gaining popularity among growers is the use of very simple tanks in situ to apply it immediately after production. However, this production system is very basic and does not easily permit adequate quality control of the inoculum.

An alternative system for production of inoculum is solid fermentation. In this case, agricultural waste materials such as rice and wheat straw, sugarcane bagasse, ground corncobs, sawdust, rice and wheat bran are used as either food base or substrate alone or in combination to grow *Trichoderma*. When using solid fermentation to produce the inoculum, the food base in the formulation should favor the bioinoculant (Papavizas et al., 1984) or even better, a food base that can only be utilized by desired microorganisms is strongly recommended (Nelson and Powelson, 1988). This method of formulation requires minimal cost and small scale production but it is bulky and requires large space for production, inoculation and storage including drying and milling. Furthermore, from an engineering point of view, control of pH and nutrient availability in solid fermentation systems is more complex, impacting quality control of the product. In both solid and liquid fermentation systems, it is required to dry the product to achieve higher stability and longer shelf life (Jin et al., 1992). Drying is essential in order to prevent spoilage by microbial contamination but results in losses of viability during the process, particularly when carried out at elevated temperatures (Jin and Custis, 2011). A dry product has the advantage of convenient storage and transport. Spray drying is perhaps the method of choice among the different drying techniques for large scale production due to its low cost (Morgan et al., 2006).

Microencapsulation of conidia with sugars, such as sucrose, trehalose, molasses or glycerol increases the survival of *Trichoderma* (Jin and Custis, 2011). Microencapsulation is defined as a process in which very small particles or droplets are surrounded by a protective coat, or embedded in a homogeneous or heterogeneous matrix, to produce small capsules (Gharsallaoui et al., 2007). This process results in prolonged shelf life and controls microbial release, thus enhancing its application efficiency (John et al., 2011; Rathore et al., 2013). Microencapsulation provides living cells with a physical barrier that protects them from adverse environmental conditions (O'Riordan et al., 2001; Ross et al., 2005). For instance, the use of a maltodextrin-gum biopolymer matrix for microencapsulation of *T. harzianum* resulted in high conidia survival after spray-drying, 11-fold higher than non-encapsulated conidia (Muñoz-Celaya et al., 2012).

Some additives including protectants and carriers have been used to increase fungal survival under adverse environmental conditions, including fine clay, peat, vermiculite, alginate, wheat, bran, talc, diatomaceous earth, corn and sugarcane bagasse and pasteurized soil (Martínez-Medina et al., 2009; Doni et al., 2014). A study used bentonite–vermiculite as a solid substrate for both *T. harzianum* proliferation and as a carrier based on the consideration that both are harmless to the environment, low cost, and easily available. It investigated the survival and effectiveness of *T. harzianum* bentonite–vermiculite formulation and its growth promotion on melon plants under nursery conditions compared with the incorporation of this agent as conidia suspension (Martínez-Medina et al., 2009). The effectiveness of the application of *T. harzianum* to plants was related directly to its formulation, since the level of *T. harzianum* colony-forming units lasted after 8 weeks, whereas the supply of liquid suspension reduced it by two orders of magnitude. Plants treated with the bentonite–vermiculite formulation showed a higher shoot weight than untreated plants. The effectiveness of other carriers of *Trichoderma* sp. SL2, including ground corn or sugarcane bagasse on rice seedling growth was

also tested (Doni et al., 2014). The use of potato dextrose agar as carrier was not practical for field application due to its short shelf life and high cost. When a *Trichoderma* sp. SL2 suspension mixed with ground corn was used as a treatment supplied to soil, it significantly enhanced rice root length, fresh weight and total biomass as compared to the mixture with sugarcane bagasse or the control, indicating its potential application as carrier for *Trichoderma* spp. inoculants. In another study, greater dry weight of radish shoots was evidenced when *Trichoderma* was applied to compost as a peat–bran inoculum in comparison to its supply as suspension of conidia (Baker et al., 1984). Similar observations were made by Kleifeld and Chet (1992) who found that a peat–bran preparation of *T. harzianum* T-203 was more effective in promoting plant growth in pepper and other plants than conidial suspensions or seed coatings. Importantly, in the peat–bran inoculated plants *Trichoderma* proliferated on roots for over 25 days, suggesting that the peat–bran could be used as a food reservoir for *Trichoderma*. Nevertheless, Bell et al. (2000) reported a contrasting effect on the presence nutrients in a pellet formulation of *T. harzianum*, which inhibited growth of cucumber seedlings, whereas a seed coat formulation stimulated growth, leading the authors to suggest that the food source included in the pellets could have supported growth of inhibitory microbes in the soil.

Application of *Trichoderma* inoculum at early stage of crop growth permits to maximize its benefits in terms of root development and nutrient uptake. Increases in plant growth following *Trichoderma* treatment depend on the specific crop or plant genotype. For example, in a series of repeated trials, six strains of *Trichoderma* consistently promoted growth of lettuce (Ousley et al., 1994). However, when Baker (1988) tested the same isolate on pea and radish, he found only small increases in growth. In a more recent study, Tucci et al. (2011) showed that genetic variability among wild and cultivated tomato varieties affects the outcome of the interaction with two strains of *T. atroviride* and *T. harzianum*. The expected beneficial response, which included enhanced growth, was obtained for some, but not all tested varieties. Furthermore, at least in one case, treatment with *Trichoderma* had no effect or resulted even detrimental on crop growth. A simple explanation for the lack of beneficial effects is that the isolates differ in their capacity to colonize certain plant roots. However, it can not be discarded that the promotion or inhibition effects exhibited by the same strain in different interactions may be due to differential production of secondary metabolites, some of which may be detrimental to certain plant genotypes, and that may be the result of the *Trichoderma*-plant metabolic crosstalk.

Successful results do not only rely on the use of an effective bioinoculant but also on the method of delivery or application on the seed, root and soil. In this sense, either seed treatment or coating, are highly recommended and effective methods of application of *Trichoderma* for agricultural purposes (Mathre et al., 1999). It is preferable to apply in advance *Trichoderma* into the soil to build up a *Trichoderma* population, which may provide synergic effects with other agronomical practices such as organic fertilization. In contrast, soil fumigation or fungicide application may negatively affect native or incorporated *Trichoderma* populations.

4. Effects of *Trichoderma* on growth, nutrient uptake and stress tolerance of crops

4.1. Plant growth

Trichoderma improves plant growth and development in axenic systems, in greenhouses, or in the field (Table 1). Several reports have shown the beneficial effects of *Trichoderma* spp. on horticultural crops such as cucumber, periwinkle, chrysanthemum and lettuce on seed germination, vegetative growth and flowering (Chang et al., 1986; Hermosa et al., 2012; Studholme et al., 2013). Cucumber seedlings grown in soil amended with *T. harzianum* propagules sustained a 30% increase in seedling emergence 8 days after sowing. Three weeks later, these plants exhibited a 75% and 95% increase in root length and total root area, respectively, and substantial increases in dry weight, shoot length and leaf area (Yedidia et al., 2001). This early report showed correlation between increased root growth and shoot biomass production, which was further observed in crops such as maize, bean and tomato (Björkman et al., 1998; Björkman, 2004; Harman, 2011; Vargas et al., 2009; Azarmi et al., 2011; Pereira et al., 2014). *Trichoderma* isolates including *T. harzianum* isolate T-969, *T. harzianum* isolate T-447 and *Trichoderma* sp. isolate T were supplied either directly to tomato seeds or to nursery soil. Seed germination rate was not affected by *Trichoderma* application, whereas shoot height, shoot diameter, shoot fresh and dry weights were increased by *Trichoderma* sp. T and *T. harzianum* T-969. Plants grown on soil amended with *Trichoderma* sp. T and *T. harzianum* T-969 had also a marked increase in leaf number, leaf area and chlorophyll content (Azarmi et al., 2011). The interaction between *T. harzianum* CECT 2413 strain and the tomato-root system was also studied during the early stages of root colonization by the fungus. Inoculation of *T. harzianum* conidia into the liquid medium of hydroponically grown tomato plants caused profuse adhesion of hyphae to the roots as well as colonization of the root epidermis and cortex. Confocal microscopy of a *T. harzianum* strain that expressed the green fluorescent protein (GFP) revealed intercellular hyphal growth and the formation of plant-induced papilla like hyphal tips. Analysis of the *T. harzianum*–tomato interaction in soil indicated that the contact between the fungus and roots persisted over a long period of time (Chacón et al., 2007). *T. parareesei* and *T. reesei*, which produce cellulases and xylanases of industrial interest also increased lateral root development and acclimatized tomato plants to grow under salt stress conditions (Rubio et al., 2014).

Propagation *in vitro* of cuttings is important for many horticultural crops. Increased vigor of rooted cuttings results in optimized propagation with reduced fungicide inputs, improved quality and faster stock turnout. Adams et al. (2007) compared the effects of *T. harzianum* Rifai 1295-22, also known as 'T-22' and commercial formulations of ectomycorrhizal fungi in the establishment and growth of crack willow (*Salix fragilis* L.). Tree samplings grown with *T. harzianum* T-22 produced shoots and roots that were 40% longer than those of the controls and shoots that were 20% longer than those of samplings grown with ectomycorrhiza. Moreover, *T. harzianum* T-22 samplings produced more than double the dry biomass of controls and more than 50% extra biomass than the ectomycorrhiza treated samplings. In another work, the potential of 62 *Trichoderma* isolates to enhance root development on cuttings was evaluated in a screening bioassay using the ornamental plant *Impatiens walleriana* (Clouston et al., 2010). Six individual *Trichoderma* isolates and a commercial mixture of isolates were identified, which improved root length, root dry weight, shoot dry weight, and root/shoot ratio when compared to untreated cuttings. Sofo et al. (2010) tested the effects of *Trichoderma* T-22 on shoot growth and rooting of GiSeLa6 (*Prunus cerasus* × *P. canescens*) and of GF677 (*P. amygdalus* × *P. persica*), cultured *in vitro*. The results showed that early inoculation of the fungus at the stage of shoot transfer to root-inducing medium damaged both GiSeLa6 and GF677 plants, whereas, following later inoculation (7 d after shoot transfer to root-inducing medium), the plants survived and showed significant increases in shoot growth and root development. In particular, root lengths in GiSeLa6 and GF677 plants increased by 180% and 136%, respectively, compared to non-inoculated controls. These morphological characteristics could increase the quality and viability of nursery planting material and provide advantages during the plant

Table 1
Biostimulant effects of *Trichoderma* applications on agronomical and physiological aspects of horticultural crops, and *Arabidopsis thaliana*.

Crop	*Trichoderma* species/strain	Application mode	Experimental conditions	Effects	Reference
Arabidopsis	*Trichoderma virens* and *Trichoderma atroviride*	Seedling were grown in Petri dishes and inoculated with 1×10^6 conidia	*In vitro*	Increased lateral root formation and biomass production	Contreras-Cornejo et al. (2009)
Arabidopsis	*Trichoderma hamatum* strain GD12	*Trichoderma* bran inoculum added to soil before sowing	Microcosms	Promoted root and shoot growth	Studholme et al. (2013)
Arabidopsis	*Trichoderma asperelloides* T203	Root system was inoculated with a solution containing 1×10^5 spores[a]/ml	Hydroponics	Improved seed germination	Brotman et al. (2013)
Arabidopsis	*Trichoderma virens* Tv29.8 and *Trichoderma atroviride* IMI 206040	Seedling were grown in Petri dishes and inoculated with 1×10^6 conidia	*In vitro*	Improved plant growth under saline conditions, induced lateral root and root hair formation	Contreras-Cornejo et al. (2014b)
Arabidopsis	*Trichoderma virens* and *Trichoderma virens Δppt1*	Seedling were grown in divided Petri dishes and exposed to fungal volatiles emitted from a colony developed from 1×10^6 conidia	*In vitro*	Increased lateral root formation and growth	Contreras-Cornejo et al. (2014c)
Bean	*Trichoderma harzianum* (ALL 42)	Seeds were immersed in a spore suspension containing 2.4×10^8 conidia/ml	*In vitro*	Increased overall plant size and the number of lateral roots	Pereira et al. (2014)
Cherry	*Trichoderma harzianum* T-22	Plants were inoculated with approximately 50,000 conidia	*In vitro*	Increased shoot growth and root development	Sofo et al. (2010)
Chickpea	*Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma virens*	The growth medium or seeds were inoculated with a talc-based formulation of ground mycelium	*In vitro* glasshouse and field conditions	Increased shoot branching, biomass, grain yield and P and N uptake	Rudresh et al. (2005)
Chickpea	*Trichoderma* sp.	Agar plates were inoculated with a fungal mycelial disc of 5 mm diameter	*In vitro*	Increased solubilization of inorganic phosphate	Rawat and Tewari (2011)
Chrysanthemum	*Trichoderma harzianum*	5×10^6 conidia per g of soil or sprayed on roots at a concentration of 1×10^8 conidia/ml	Greenhouse	Promoted seed germination, vegetative growth and flowering	Chang et al. (1986)
Cucumber	*Trichoderma harzianum*	5×10^6 conidia per g of soil or sprayed on roots at a concentration of 1×10^8 conidia/ml	Greenhouse	Promoted seed germination, vegetative growth and flowering	Chang et al. (1986)
Cucumber	*Trichoderma harzianum* T-203	Mycelial inoculum added directly to the nutrient solution and applied to the soil	Hydroponics and greenhouse	Increased root length, total root area, shoot length and leaf area	Yedidia et al. (2001)
Cucumber	*Trichoderma harzianum*	Plants grown in Petri dishes were inoculated with 1×10^6 spores. Seeds were coated with a spore suspension containing 2×10^8 spores[a]/ml	*In vitro* hydroponics greenhouse	Increased the length of root hairs and lateral roots	Samolski et al. (2012)
Cucumber	*Trichoderma asperelloides* T203	Root system was inoculated with a solution containing 1×10^5 germinated spores[a]/ml	Hydroponics	Improved seed germination	Brotman et al. (2013)
Lettuce	*Trichoderma hamatum* strain GD12	*Trichoderma* bran inoculum added to soil before sowing	Microcosms	Promoted root and shoot growth	Studholme et al. (2013)
Lettuce	*Trichoderma atroviride* MUCL 45632	The substrate was supplied with prepared tablets containing 4.5×10^5 conidia	*In vitro* greenhouse, and field conditions	Enhanced shoot and root dry weight, and chlorophyll content	Colla et al. (2015a)

Table 1 (*Continued*)

Crop	*Trichoderma* species/strain	Application mode	Experimental conditions	Effects	Reference
Maize	*Trichoderma harzianum* 1295-22	Seeds were treated with a suspension of 5×10^6 spores[a]	Greenhouse	Increased root and shoot growth	Björkman et al. (1998)
Maize	*Trichoderma harzianum* T-22	Seeds were treated with a commercial formulation, containing 1×10^9 CFU (primarily conidia)	Greenhouse	Induced root and shoot growth, increased plant biomass	Harman et al. (2004)
Melon	*Trichoderma harzianum*	Solid bentonite–vermiculite formulation containing 1×10^9 conidia/g	Nursery conditions	Increased plant growth and shoot biomass	Martínez-Medina et al. (2009)
Melon	*Trichoderma atroviride* MUCL 45632	The substrate was supplied with prepared tablets containing 4.5×10^5 conidia	*In vitro* greenhouse, and field conditions	Enhanced shoot and root dry weight, and chlorophyll content	Colla et al. (2015a)
Pepper	*Trichoderma harzianum*	5×10^6 conidia per g of soil or sprayed on roots at a concentration of 1×10^8 conidia/ml	Greenhouse	Promoted seed germination, vegetative growth and flowering	Chang et al. (1986)
Pepper	*Trichoderma atroviride* MUCL 45632	The substrate was supplied with prepared tablets containing 4.5×10^5 conidia	*In vitro* greenhouse, and field conditions	Enhanced shoot and root dry weight, and chlorophyll content	Colla et al. (2015a)
Periwinkle	*Trichoderma harzianum*	5×10^6 conidia per g of soil or sprayed on roots at a concentration of 1×10^8 conidia/ml	Greenhouse	Promoted seed germination, vegetative growth and flowering	Chang et al. (1986)
Tomato	*Trichoderma harzianum Rifai T-22*	Seeds were treated with a conidial suspension of 2×10^7 CFU per g of seed	*In vitro*	Promoted seed germination and growth	Mastouri et al. (2010)
Tomato	*Trichoderma harzianum* and *Trichoderma* sp.	Seeds were immersed in 1 ml of suspension containing 10^6–10^7 spores[a] per ml	Greenhouse	Promoted shoot and root growth, and increased leaf number, leaf area and chlorophyll content	Azarmi et al. (2011)
Tomato	*Trichoderma harzianum*	Plants grown in Petri dishes were inoculated with 1×10^6 spores. Seeds were coated with a spore suspension containing 2×10^8 spores[a]/ml	*In vitro* Hydroponics, greenhouse	Increased the length of root hairs and lateral roots	Samolski et al. (2012)
Tomato	*Trichoderma parareesei*	Plants were grown in Petri dishes and inoculated with 1×10^6 conidia	*In vitro*	Increased lateral root development and promoted growth	Rubio et al. (2014)
Tomato	*Trichoderma atroviride* MUCL 45632	The substrate was supplied with prepared tablets containing 4.5×10^5 conidia	*In vitro* greenhouse, and field conditions	Enhanced shoot and root dry weight, and chlorophyll content	Colla et al. (2015a)
Zucchini	*Trichoderma atroviride* MUCL 45632	The substrate was supplied with prepared tablets containing 4.5×10^5 conidia	*In vitro* greenhouse, and field conditions	Enhanced shoot and root dry weight, and chlorophyll content	Colla et al. (2015a)

[a] Authors do not specify the type of spores used (presumably conidia).

acclimatization phase. Thus, selected strains of *Trichoderma* may be used to develop plant growth promotion products that fasten the rooting process and improve cutting establishment for horticultural species.

4.2. Plant nutrition

Intensive agriculture demands an adequate supply of nutrients, which act as structural components of cells or play roles in photosynthesis and metabolism. At the rhizosphere, both macronutrients and micronutrients undergo a complex dynamic equilibrium of solubilization, uptake and transport that is greatly influenced by root associated microorganisms. In particular, N and P can directly act as signals that alter post-embryonic root development, modifying primary and lateral root growth and root hair formation and may be involved in the success of plant-microbe associations (Giehl and von Wirén, 2014; Ruiz-Herrera et al., 2015).

Accumulating information has shown the efficacy of *Trichoderma* spp. as biofertilizers, since their application to soil, seeds or plant surfaces increases the solubility of nutrients as well as the nutrient uptake capacity of the root and/or their distribution within plant parts. These beneficial properties are explained *via* modulation of root architecture or through the exudation of substances that increase nutrient availability such as siderophores and organic acids (Samolski et al., 2012; Zhao et al., 2014). The importance of root architectural alterations induced by *Trichoderma* in plant nutrition was evident following the early work by Björkman et al. (1998). This report showed the correlation between increased root growth and shoot biomass production in maize, which was investigated in detail later in *Arabidopsis* and cucumber plants (Contreras-Cornejo et al., 2009; Samolski et al., 2012). *T. virens* and *T. atroviride* promoted *Arabidopsis* seedling growth increasing root and shoot biomass production *via* prolific formation of lateral roots (Contreras-Cornejo et al., 2009; Fig. 2C and D). Inoculation of cucumber roots with a *T. harzianum* strain that overexpresses the qid74 gene, which encodes a cysteine-rich cell-wall protein, increases root hair initiation and growth, whereas fewer and shorter root hairs developed in roots inoculated with a strain defective on qid74. Modifications in root architecture in cucumber plants induced by WT or qid74 overexpressing strains increased plant biomass through an efficient use of N, P, K and micronutrients (Samolski et al., 2012).

Data showing the increase of nutrient content in horticultural and crop species as a result of *Trichoderma* inoculation confirm the biostimulation properties of these fungi. *T. harzianum* 1295-22 increased the availability of P and several micronutrients such as Fe, Mn and Zn in liquid sucrose–yeast extract medium *in vitro* (Altomare et al., 1999). *T. harzianum* strain T-203 (later on identified as *Trichoderma asperellum* and recently as *Trichoderma asperelloides*) increased 90% and 30% the P and Fe concentration in roots, respectively. An increased growth response was apparent as early as 5 days post-inoculation, resulting in an increase in root and shoot biomass production with a concomitant elevation in the concentration of Cu, P, Fe, Zn, Mn and Na in inoculated roots (Yedidia et al., 2001).

Phosphate solubilization is a major beneficial trait of *Trichoderma* regarding the depletion of phosphate mines and the concomitant increase in P fertilizer prices. Rudresh et al. (2005a) reported the ability of nine isolates of *Trichoderma* spp. to solubilize calcium phosphate as compared with an efficient phosphate-solubilizing bacterium *Bacillus megaterium* subsp. *phosphaticum* PB that was used as the reference strain. *T. viride* (TV 97), *T. virens* (PDBCTVs 12) and *T. virens* (PDBCTVs 13) released 70% more P than the reference bacterial strain. Pot culture and field evaluations further demonstrated that *T. harzianum* (PDBCTH 10), *T. viride* (TV 97), and *T. virens* (PDBCTVs 12) increased P uptake in chickpea (*Cicer arietinum* L.) plants supplied with rock-phosphate as P source, which correlated with growth and yield parameters. *T. harzianum* retained its P solubilizing potential at varying concentrations of cadmium, indicating that *Trichoderma* may provide advantages to plants even in soils polluted with heavy metals (Rawat and Tewari, 2011). In another study, *T. harzianum* isolate T-969, increased the concentrations of Ca, Mg, P and K compared with the control, with positive effects on shoot height, shoot diameter, and shoot fresh and dry weights in tomato seedlings (Azarmi et al., 2011). An interesting possibility, still to be investigated, is that *Trichoderma* or its metabolites could up-regulate the expression of nutrient transporters in plants roots, thus improving nutrient uptake efficiency.

Iron (Fe) deficiency is an agriculturally important problem in alkaline soils due to the insolubility of Fe forms. To investigate the potential of *Trichoderma* to solubilize Fe in the rhizosphere, a high siderophore-producing strain of *T. asperellum* (T-6) was isolated from cucumber roots. Applying strain T-6 to sterilized soil could increase soil levels of Fe^{2+} and siderophores, as well as increase Fe^{2+} and Fe^{3+}-chelate reductase activity in cucumber roots (Zhao et al., 2014). Extracellular Fe^{3+} reducing activity and organic acid release were evidenced upon analysis of the culture filtrate of T-6. Moreover, Colla et al. (2015a) demonstrated that *T. atroviride* MUCL45632 was able to produce two types of siderophores: hydroxamate and catechol compounds. These results indicate that *Trichoderma* influences Fe nutrition of crops. Exudation of organic acids and release of Fe^{3+} reducing enzymes may contribute in the solubilization and reduction of insoluble Fe^{3+} to the more soluble Fe^{2+} boosting the root Fe uptake capacity.

The improvements of *Trichoderma*-based biostimulants may help farmers reduce the use of fertilizers and develop novel strategies to optimize fertilizer use efficiency. With this aim, Molla et al. (2012) tested the ability of *Trichoderma* spp. to increase growth of tomato plants when supplied together with fertilizer. It was found that supplementation of fertilizer with *Trichoderma* enhanced plant production by 50% compared with a standard dose of N, P, and K macronutrients, minimizing the use of fertilizer and their potential negative effects in the environment.

4.3. Abiotic stress tolerance

Heat, drought, cold, and salinity are the major abiotic stresses that induce severe cellular damage in crop plants. Fungal species are commonly found in association with plant communities resisting naturally to different types of stress (Marasco et al., 2012). *Trichoderma* colonized plants display an in increased endogenous level of auxins, ethylene and gibberellins, plant enzymes, antioxidants and compatible solutes and compounds like phytoalexins and phenols that provide tolerance to environmental stress. Since ethylene is considered a plant growth regulating compound that inhibits root development and plant growth (Lewis et al., 2011), its production by fungi or in plants in response to fungal root colonization may be important to fine-tune induced biotic or abiotic stress responses.

4.3.1. Salt tolerance

The beneficial effects of *Trichoderma* species on alleviating the adverse effects of salt stress have been recently documented in *Arabidopsis* and crop plants (Mastouri et al., 2010, 2012; Rawat et al., 2013; Contreras-Cornejo et al., 2014b; Hashem et al., 2014). Mastouri et al. (2010) reported that *T. harzianum* T-22 treated seeds germinated faster and more uniformly than untreated seeds whether the stress applied was osmotic, salt, or suboptimal temperatures. Treatments with the antioxidant glutathione, or application of T-22, resulted in a similar protective effect. These findings support the notion that *T. harzianum* strain T-22 increases seedling vigor during osmotic stress by inducing physiological responses of plants against oxidative damage. Application of *T. hamatum* to

Ochradenus baccatus plants grown under standard conditions stimulated plant growth. Salt treatments that increase salt levels in plant tissues decreased growth, pigment content, membrane stability, water content, and total lipid content (Hashem et al., 2014). These effects were explained because salt stress causes lipid peroxidation and thus adaptive responses mediate plant survival *via* biosynthesis of phenols, diacylglycerol, sterol esters, nonesterified fatty acids, and activation of antioxidant and ROS detoxifying enzymes. *T. hamatum* alleviated the antagonistic effect of salinity on plant growth and metabolic processes.

Arabidopsis and cucumber plants treated with *Trichoderma asperelloides* prior to salt stress imposition showed significantly improved seed germination (Brotman et al., 2013). An evaluation of *Arabidopsis* roots inoculated with T-203 by microarray analysis coupled with qPCR studies revealed wide gene transcript reprogramming, involving genes related to osmo-protection and response to oxidative stress. The monodehydroascorbate reductase (MDAR), and superoxide dismutase (SOD) genes were up-regulated in *Arabidopsis* and cucumber, respectively, while an increase in the pool of ascorbic acid in plant hosts contributed to the osmoprotectant properties of *Trichoderma*.

Auxin signaling is a major target of salinity stress in plants (Zörb et al., 2013). Auxin production by *T. virens* (Tv29.8) and *T. atroviride* (IMI 206040) promoted plant growth in both normal and saline conditions, which was related to altered root architecture and biochemical changes. *Arabidopsis* seedlings grown under saline conditions inoculated with *Trichoderma* spp. showed increased levels of abscisic acid, L-proline, and ascorbic acid, and enhanced elimination of Na^+ through root exudates (Contreras-Cornejo et al., 2014b). These data show the critical role of auxin signaling and root architecture to salt tolerance in *Arabidopsis* and suggest that these fungi may enhance the plant IAA level as well as the antioxidant and osmoprotective status of plants under salt stress.

4.3.2. Drought tolerance

Trichoderma spp. may enhance drought tolerance to plants *via* improved root development (Contreras-Cornejo et al., 2009; Shukla et al., 2012), activating antioxidant protection against damage by dehydration (Mastouri et al., 2012; Brotman et al., 2013), and/or delaying drought induced changes in stomatal opening, photosynthesis and chlorophyll leaf content. The tolerance to water deficit of tomato seedlings was attributed to activation of antioxidant responses and higher activity of ascorbate and glutathione-recycling enzymes (Mastouri et al., 2012). Bae et al. (2009) reported that the total amino acid (histidine, arginine, proline, c-aminobutyric acid, valine, and leucine) content increased in response to drought in untreated cacao plants, but not in *T. hamatum* DIS 219b-colonized seedlings, suggesting that amino acid accumulation may be a response to drought. Thus, *Trichoderma* diminished the negative effects of stress on plant metabolism. Shukla et al. (2012) showed that the primary direct effect of *Trichoderma* colonization in rice was promotion of root growth, regardless of water status, which caused delay in the drought responses of rice plants. Five *Trichoderma* isolates that were resistant to drought were identified, which delayed drought induced changes like stomatal conductance, net photosynthesis and leaf greenness. Drought conditions varying from 3 to 9 days of withholding water led to an increase in the concentration of many stress induced metabolites in rice leaves, while *Trichoderma* colonization caused a decrease in proline, MDA and H_2O_2 contents, and increased phenolic compound concentration (Shukla et al., 2012). Contreras-Cornejo et al. (2015a) investigated the potential of *T. virens* and *T. atroviride* in modulating stomatal aperture and plant transpiration. *Arabidopsis* wild type (WT) seedlings and ABA-insensitive mutants *abi1-1* and *abi2-1* were co-cultivated with these fungi under well watered conditions and stomatal aperture and water loss determined in leaves. *Arabidopsis* WT seedlings inoculated with *Trichoderma* showed both decreased stomatal aperture and reduced water loss when compared to uninoculated seedlings. This effect was absent in *abi1-1* and *abi2-1* mutants. *T. virens* and *T. atroviride* induced the abscisic acid inducible marker *abi4:uidA* and produced ABA, which might be involved together with auxins, in stress tolerance mechanisms. All together, these reports show a novel facet of *Trichoderma* in boosting plant health under drought stress. Moreover, fungal metabolites can be useful toward regulating stomatic aperture and water use efficiency of plants.

5. Molecular mechanisms of *Trichoderma* phytostimulation

Arabidopsis thaliana has been established as an excellent model system to study the genetic and physiological mechanisms by which *Trichoderma* promotes plant growth. This has been possible because of the vast knowledge gained from physiological processes and genetic pathways on this small weed, and the ability to test the *Trichoderma*-plant interaction under axenic conditions (Contreras-Cornejo et al., 2009, 2011). With the advent of genomics revolution, other crops such as maize (Shoresh and Harman, 2008; Vargas et al., 2009, 2011; Harman, 2011), tomato (Rubio et al., 2012) and bean (Pereira et al., 2014) have been used to advance the existing knowledge about the genes and proteins mediating the phytostimulation process.

Contreras-Cornejo et al. (2009) investigated in detail the role of auxin signaling in *Trichoderma*–plant interactions in *Arabidopsis thaliana*. The authors found that mutations in genes involved in auxin transport or signaling, *AUX1, BIG, EIR1* and *AXR1*, reduced the beneficial effects of *Trichoderma* on biomass production and root branching. Colonization of roots induced the auxin response, which correlated with increased development of lateral roots. The application of three identified indolic compounds to *Arabidopsis* seedlings showed a dose-dependent effect on biomass production, increasing yield in small amounts (nM range) but repressing growth at higher concentrations (mM range). For example, the application of indole-3-carboxaldehyde to *Arabidopsis* seedlings inhibited primary root growth while inducing adventitious root formation (Contreras-Cornejo et al., 2011). The connection between fungal produced auxin and root development elicited by *Trichoderma* was found to depend on mitogen activated protein kinase signaling (Contreras-Cornejo et al., 2015b). Inoculation of *Arabidopsis* with *T. atroviride* regulates root growth and root hair formation modulating MPK6 activity, likely depending on ethylene (ET) and auxin signaling. It was found that ET, and indole-3-acetic acid (IAA) produced by the fungus induce MPK6 activity. Further analysis of auxin-related gene expression in WT seedlings and ethylene-related mutants showed that the effect of ET on root growth and differentiation apparently is mediated by auxin signaling, thus indicating that an auxin-ethylene crosstalk involving MPK6 fine-tunes seedling growth and development in response to a beneficial soil fungus. In consequence, MPK6, and its MAP kinase associated cascade, whose components remain to be identified, seems to be a regulation node to maintain and/or amplify the hormonal effects underlying plant development.

Root colonization with *T. harzianum* (T-22) induced changes in the proteome of shoots of maize seedlings. Using a proteomics strategy, proteins that are involved in carbohydrate metabolism, photosynthesis or development were identified likely mediating the maize response to *Trichoderma* inoculation. 16 up-regulated proteins were related to DNA metabolism and genetic information processing including transcription factors and nuclear proteins such as RNA polymerase I, II, and III, RNA-binding and nuclear proteins, a BTB/POZ domain-containing protein, splicing factor SC35, FCP1-like phosphatase, and a DNA repair-recombination protein, RAD50 (Shoresh and Harman, 2008). This suggests that the differences observed in plants biostimulated with *Trichoderma* require

the involvement of regulatory proteins. Down regulated proteins included a β-glucosidase protein, which putatively hydrolyzes cytokinin–conjugates and releases free cytokinins during plant growth and development and a small peptide (DVL protein) found to affect *Arabidopsis* development (Shoresh and Harman, 2008).

T. harzianum (ALL-42), isolated from Brazilian Cerrado soil promoted common bean growth, overall size, foliar area, root area, and the number of lateral roots in comparison to plants grown without the fungus. A proteomic analysis revealed changes in protein profiles with potential regulatory functions including a histone acetyl-transferase complex component, and NAC1 domain protein. The transcription factors classified as NAC family members play a crucial role in a wide range of plant developmental processes, such as lateral root development. Changes in protein metabolism were also observed in the root proteomics maps, up-regulated for common bean plants challenged with phytopathogenic fungi and *T. harzianum*. An increase in fructokinase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ferritin, which play a crucial role in carbohydrate metabolism, as well as iron nutrition, explains some of the biofertilizer properties of this fungus. Ferritin was highly expressed in the proteomic maps of *Trichoderma*-treated plants, suggesting an additional role in increasing the ability of plants to mobilize and transport iron (Pereira et al., 2014).

6. Bioactive compounds from *Trichoderma*

6.1. Auxins

Auxin signaling plays a role in nearly every aspect of plant development from embryogenesis to reproduction. Signals from the developmental program, physiological status, and encounters with root microorganisms all converge on the auxin pathway (Ortiz-Castro et al., 2011; Kazan, 2013; Simon et al., 2013). The potential of plant-associated microorganisms to produce free IAA, auxin precursors or auxin signal mimics influence the endogenous auxin response of the host and are important modulators of root system architecture (Contreras-Cornejo et al., 2009; Felten et al., 2009; Hilbert et al., 2012).

T. virens and *T. atroviride* produce a variety of molecules with auxin activity as part of their metabolism including IAA, indole-3-ethanol, indole-3-acetaldehyde and indole-3-carboxaldehyde (Contreras-Cornejo et al., 2009, 2011). Following the hypothesis that direct plant growth-promoting activity of *T. harzianum* strain T-22 could depend on changes in the levels of different classes of plant growth regulators, Sofo et al. (2011) analyzed phytohormone levels in cherry rootstocks (*Prunus cerasus* × *P. canescens*) inoculated with this fungal strain. Root and shoot growth increased by 76 and 61%, respectively in plants treated with T-22, which correlated with 49 and 40% higher levels of IAA in both leaves and roots. In tomato plants, the phytostimulant properties of *Trichoderma* spp. also depended on auxin accumulation (Chowdappa et al., 2013; Martínez-Medina et al., 2014). *T. harzianum* OTPB3 strain was isolated from rhizosphere of field grown beans (*Phaseolus vulgaris* L.) and significantly increased root and shoot growth, leaf area and seedling vigor index in tomato. The level of IAA increased 45% in roots of tomato seedlings inoculated with OTPB3 when compared to uninoculated controls (Chowdappa et al., 2013). In another work, four *Trichoderma* isolates were collected from agricultural soils and identified as *T. harzianum* (two isolates), *Trichoderma ghanense*, and *Trichoderma hamatum*. Their plant growth-promoting activity on melon plants was examined *in vivo*, and compared to that of T-22. Several growth-related phytohormones were analyzed in the shoots of plants whose roots were colonized by the different *Trichoderma* isolates. An increase in auxin and a decrease in cytokinins and abscisic acid content were particular signatures of the isolates that promoted plant growth (Martínez-Medina et al., 2014). Although these later reports did not explain whether increased IAA content observed in tomato and melon originated from production by the fungi or the plant, the natural isolates might be able to produce auxin as reported for *T. virens*, alternatively, fungal colonization may up-regulate plant genes for IAA biosynthesis or down-regulate the genes involved in the catabolism of this hormone.

6.2. Secondary metabolites

Secondary metabolites comprise an heterogeneous group of natural compounds produced by plants and fungi, which aid the producing organism to survive and reinforce basic functions, such as competition or symbiosis (Demain and Fang, 2000; Bérdy, 2005). Accumulating evidence has revealed the interesting bioactivities of secondary metabolites in plant growth and development (Ramírez-Chávez et al., 2004; Vinale et al., 2008). In ecosystems, plants communicate with neighbors through volatile organic compounds (VOCs), which influence plant growth but less is known about the physiological effects of fungal VOCs on plants (Ortíz-Castro et al., 2009).

The production of secondary metabolites in *Trichoderma* spp. is strain-dependent and includes volatile and non-volatile substances, such as 6-*n*-pentyl-6H-pyran-2-one (6PP), gliotoxin, viridin, harzianopyridone, harziandione and peptaibols (Vinale et al., 2008; Reino et al., 2008). *T. atroviride* produces at least 25 different VOCs including alcohols, ketones, alkanes, furanes, pyrones, mono terpenes and sesquiterpenes (Stoppacher et al., 2010). The VOC profiles vary depending on environmental/biological growth conditions, since comparison to the control cultures revealed an inhibiting effect of cultivation of *T. atroviride* in the presence of the mycotoxin fusaric acid (FA) on the biosynthesis of 6PP, α-phellandrene, β-phellandrene, *p*-menth-2-en-7-ol and bergamotene, whereas the production of 1-octen-3-ol, 3-octanol and 3-octanone was increased in the presence of FA. Two recent reports tested the effects of *Trichoderma* VOCs on *Arabidopsis* growth and development. Hung et al. (2013) exposed plants to VOCs released by mature colonies of *T. viride* cultured on Petri plates by placing them into a growth chamber with shared atmosphere without direct physical contact. Plants grown in the presence of *T. viride* volatiles produced 45% more total biomass, had increased size, flowered earlier, and developed more lateral roots when compared to controls. Similarly, *T. virens* improved plant growth and root branching *via* emission of VOCs (Fig. 2D). GC–MS analysis of *T. viride* VOCs revealed 51 compounds of which isobutyl alcohol, isopentyl alcohol, and 3-methylbutanal were most abundant. Contreras-Cornejo et al. (2014c) examined the effects of VOCs from *T. virens* and those produced by a mutant strain defective on the 4-phosphopantetheinyl transferase 1 (TvPPT1), in plant growth promotion using divided Petri plates (Fig. 2C and D). *T. virens* produced a series of hydrocarbon terpenes, including the sesquiterpenes β-caryophyllene, (−)-β-elemene, germacrene D, τ-cadinene, δ-cadinene, α-amorphene, and τ-selinene and the monoterpenes β-myrcene, trans-β-ocimene, and cis-β-ocimene, which were absent in *Δppt1* mutant. WT and *Δppt1* fungal emissions did not affect primary root growth, but the formation of lateral roots and growth of these structures was clearly induced, indicating that such VOCs promote root branching. Other major secondary metabolites produced by *T. harzianum* and *T. atroviride* biocontrol strains have been investigated for their effect on plant growth promotion. An auxin-like activity was observed on etiolated pea stems treated with 6PP, which also promoted the growth of tomato and canola seedlings. Tomato plants sprayed with 6PP had increased total biomass and root biomass that correlated with a highly branched root system (Vinale et al., 2008).

T. harzianum produces harzianic acid (HA) and isoharzianic acid (iso-HA), a stereoisomer of HA, which promote plant growth. In a recent work, iso-HA improved germination, stem and root lengths and leaf area in tomato (Vinale et al., 2014). HPLC–DAD experiments showed that the production of HA and iso-HA was affected by the presence of plant tissue in the liquid medium. In particular, tomato tissue elicited the production of HA but negatively modulated the biosynthesis of its analogue iso-HA, suggesting that different forms of the same *Trichoderma* secondary metabolite have specific roles in the molecular mechanism regulating the *Trichoderma*–plant interaction (Vinale et al., 2014). This report indicates that HA and the new secondary metabolite iso-HA are active in plants and can be useful for agricultural applications. The identification of molecules with plant growth promoting properties can support the development of new biostimulants based on fungal compounds as active ingredients, which represent an attractive alternative to products containing only living microorganisms.

Trichoderma elicitors can be used to stimulate the production of secondary metabolites in plants (Ming et al., 2013). *Trichoderma atroviride* D16 is an endophytic fungus isolated from the root of *Salvia miltiorrhiza*, which produces various diterpene quinones termed tanshinones, which have been widely used for the treatment of cardiovascular diseases and menstrual disorders, as well as for the prevention of inflammation. The extract of mycelium (EM) and the polysaccharide fraction (PSF) isolated from *T. atroviride* D16 promoted hairy root growth and stimulated the biosynthesis of tanshinones in hairy roots. Although the active constituent responsible for these biological activities of the extracts is still unknown, these results suggest that metabolites from *T. atroviride* could elicit chemical responses in the host plant likely by influencing the expression of genes involved in the secondary metabolite biosynthesis pathway.

7. Conclusions

The use of plant beneficial fungi in agriculture has greatly increased during the past decade mostly due to their multi-level properties, and their expected success as biofertilizers (Harman, 2011; Ansari et al., 2013). Beneficial fungi with broad-host range undergo long-term interactions with a large variety of plants, thereby playing a significant role in natural and managed ecosystems and in the adaptation of crops to global climate changes (Ansari et al., 2013; Ellouze et al., 2014). *Trichoderma* spp. are promising fungi toward the development of sustainable agriculture. As such, there is an ongoing necessity to accelerate their integration in agriculture production systems, the development of successful inoculation systems and modes of delivery. The constant increase in food demand throughout the world, the rise of biofuels production and the imminent depletion of phosphate stock provisioning strengthen the necessity to seriously support research on *Trichoderma* and translate their beneficial biostimulant potential to field-grown crops.

Nutrients are taken up at the root epidermis by root hairs, a process mediated by specific transport proteins. It is noteworthy that root tips are able of sensing the local and internal concentrations of nutrients to affect root hair growth and root system architecture (Ruiz-Herrera et al., 2015). Consequently, it would be expected that the root capacity to acquire macro and micronutrients boost in the presence of microbes *via* increased nutrient solubilization and/or transport. The recent identification of transporter proteins such as the NRT1.1 nitrate transporter, which plays a key role in mediating the adaptations of roots to fluctuating levels of NO_3^- availability, but also facilitates uptake of auxin (Krouk et al., 2010) opens an interesting novel avenue for research, as the transporters may not only promote localized root growth facilitating NO_3^- influx into cells, but also modulating auxin accumulation in roots. Intriguingly, silencing of another nutrient transporter, the high-affinity ammonium transporter PiAMT1 from barley, whose transcripts accumulate during N starvation, resulted in enhanced colonization of this host by the beneficial root endophyte *Piriformospora indica* (Lahrmann et al., 2013). Determining whether *Trichoderma* metabolites including auxins and VOCs may target any nutrient transporters such as NRT1.1 or AMT1 may provide the desired combined advantages toward improving both root development and nutrient acquisition.

Biostimulated plants resulting from root colonization by *Trichoderma* spp. better resist environmental stresses such as drought and salt, and have an improved capacity to survive certain bacterial and fungal pathogen attacks. Intracellular signaling processes that translate fungal perception into genetic, metabolic and developmental responses in roots are the focus of current plant–microbe interactions research. Important signaling pathways involved in recognition of microbial elicitors, such as pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs), as well as auxin and other growth regulating substances, include mitogen-activated protein kinase (MAPK) cascades (Xu and Zhang, 2015). The recent findings that *Arabidopsis thaliana* MPK6 play a critical role in *Trichoderma* plant responses (Contreras-Cornejo et al., 2015b) open new interesting research questions, as this MAP kinase protein has been found to control plant defense responses, root development and P acquisition (Meng et al., 2013; López-Bucio et al., 2014; Lei et al., 2014). A very interesting possibility is that *Trichoderma* could enhance P uptake *via* MPK6 signaling, but it still remains to be determined whether this signaling module operates in crops and the signaling cascade acting upstream of MPK6 awaits further elucidation.

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References

Adams, P., De-Leij, F.A.A.M., Lynch, J.M., 2007. *Trichoderma harzianum* Rifai 1295-22 mediates growth promotion of crack willow (*Salix fragilis*) saplings in both clean and metal-contaminated soil. Microb. Ecol. 54, 306–313.

Altomare, C., Norvell, W.A., Björkman, T., Harman, G.E., 1999. Solubilization of phosphate and micronutrients by the plant-growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol. 65, 2926–2933.

Amsellem, Z., Zidack, N.K., Quimby Jr., P.C., Gressel, J., 1999. Long-term dry preservation of viable mycelia of two mycoherbicidal organisms. Crop Prot. 18, 643–649.

Ansari, M.W., Trivedi, K., Sahoo, R.J., Gill, S., Tuteja, N., 2013. A critical review on fungi mediated plant responses with special emphasis to *Piriformospora indica* on improved production and protection of crops. Plant Physiol. Biochem. 70, 403–410.

Atanasova, L., Le Crom, S., Gruber, S., Coulpier, F., Seidl-Seiboth, V., Kubicek, C.P., Druzhinina, I.S., 2013. Comparative transcriptomics reveals different strategies of *Trichoderma* mycoparasitism. BMC Genom. 14, 121.

Azarmi, R., Hajieghrari, B., Giglou, A., 2011. Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. Afr. J. Biotechnol. 10, 5850–5855.

Bae, H., Sicher, R.C., Kim, M.S., Kim, S.H., Strem, M.D., Melnick, R.L., Bailey, B.A., 2009. The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. J. Exp. Bot. 60, 3279–3295.

Baker, R., Elad, Y., Chet, I., 1984. The controlled experiment in the scientific method with special emphasis in biological control. Phytopathology 74, 1019–1021.

Baker, R., 1988. *Trichoderma* spp. as plant stimulants. CRC Rev. Biotechnol. 7, 97–106.

Bell, J.V., Stewart, A., Rowarth, J.S., 2000. Application method and growing medium affects the response of cucumber seedlings to inoculation with *Trichoderma harzianum*. Australas. Plant Pathol. 29, 15–18.

Benhamou, N., Chet, I., 1993. Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: ultrastructure and gold cytochemistry of the mycoparasitic process. Phytopathology 83, 1062–1071.
Benítez, T., Rincón, A.M., Limón, M.C., Codón, A.C., 2004. Biocontrol mechanisms of *Trichoderma* strains. Int. Microbiol. 7, 249–260.
Bérdy, J., 2005. Bioactive microbial metabolites. J. Antibiot. 58, 1–26.
Berg, G., 2009. Plant-microbe interactions promoting plant growth and health: perspectives for controlled used of microorganisms in agriculture. Appl. Microbiol. Biotechnol. 84, 11–18.
Björkman, T., Blanchard, L.M., Harman, G.E., 1998. Growth enhancement of *shrunken-2* sweet corn when colonized with *Trichoderma harzianum* 1295-22: effect of environmental stress. ASHS J. 123, 35–40.
Björkman, T., 2004. Effect of Trichoderma colonization on auxin-mediated regulation of root elongation. Plant Growth Regul. 43, 89–92.
Brotman, B., Landau, A., Cuadros-Inostroza, A., Takayuki, T., Fernie, A., Chet, I., Viterbo, A., Willmitzer, L., 2013. *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. PLoS Pathog. 9, e1003221.
Calvo, P., Nelson, L., Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. Plant Soil 383, 3–41.
Casas-Flores, S.E., Herrera-Estrella, A., 2007. Antagonism of plant parasitic nematodes by fungi. In: Kubicek, C.P., Druzhinina, I.S. (Eds.), The Mycota, Environmental and Microbial Relationships, 4, second ed. Springer, Germany, Berlin, Heidelberg, U.S.A. New York, pp. 159–187.
Chacón, M.R., Rodríguez-Galán, O., Benítez, T., Sousa, S., Rey, M., Llobell, A., Delgado-Jarana, J., 2007. Microscopic and transcriptome analyses of early colonization of tomato roots by *Trichoderma harzianum*. Int. Microbiol. 10, 19–27.
Chang, Y.C., Baker, R., Klefield, O., Chet, I., 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Dis. 70, 145–148.
Chandanie, W.A., Kubota, M., Hyakumachi, M., 2009. Interactions between the arbuscular mycorrhizal fungus *Glomus mosseae* and plant growth-promoting fungi and their significance for enhancing plant growth and suppressing damping-off of cucumber (*Cucumis sativus* L.). Appl. Soil Ecol. 41, 336–341.
Chaverri, P., Gazi, R., Samuels, G.J., 2011. *Trichoderma amazonicum*, a new endophytic species on *Hevea brasiliensis* and *H. guianensis* from the Amazon basin. Mycologia 103, 139–151.
Chowdappa, P., Mohan Kumar, S.P., Lakshmi, M.J., Upreti, K.K., 2013. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biol. Control 6, 109–117.
Churchill, B.W., 1982. Mass production of microorganisms for biological control. In: Charudattan, R., Walker, H.L. (Eds.), Biological Control of Weeds with Plant Pathogens. John Wiley and Sons, New York, pp. 139–156.
Clouston, A.M., Hill, R.A., Minchin, R., Braithwaite, M., Stewart, A., 2010. A bioassay screening *Trichoderma* isolates for enhancement of root development in *Impatiens walleriana* cuttings. N. Z. Plant Prot. 63, 33–38.
Colla, G., Rouphael, Y., Di Mattia, E., El-Nakhel, C., Cardarelli, M., 2015a. Co-inoculation of *Glomus intraradices* and *Trichoderma atroviride* acts as a biostimulant to promote growth, yield and nutrient uptake of vegetable crops. J. Sci. Food Agric. 95, 1706–1715.
Colla, G., Rouphael, Y., Bonini, P., Cardarelli, M., 2015b. Coating seeds with endophytic fungi enhances growth nutrient uptake, yield and grain quality of winter wheat. Int. J. Plant Prod. 9, 171–190.
Contreras-Cornejo, H., Macías-Rodríguez, L., Cortés-Penagos, C., López-Bucio, J., 2009. *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. Plant Physiol. 149, 1579–1592.
Contreras-Cornejo, H.A., Macías-Rodríguez, L., Beltrán-Peña, E., Herrera-Estrella, A., López-Bucio, J., 2011. *Trichoderma*-induced plant immunity likely involves both hormonal and camalexin-dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungi *Botrytis cinerea*. Plant Signal. Behav. 6, 1554–1563.
Contreras-Cornejo, H.A., Ortiz-Castro, R., López-Bucio, J., 2013. Promotion of plant growth and the induction of systemic defence by *Trichoderma*: physiology, genetics and gene expression. In: Mukherjee, P., Horwitz, B.A., Singh, U.S., Mukherjee, M., Schmoll, M. (Eds.), *Trichoderma*: Biology and Applications. CAB International, pp. 175–196.
Contreras-Cornejo, H.A., Macías-Rodríguez, L.I., López-Bucio, J.S., López-Bucio, J., 2014. Enhanced plant immunity using *Trichoderma*. In: Gupta, V.K., Schmoll, M., Herrera-Estrella, A. (Eds.), Biotechnology and Biology of Trichoderma. , pp. 495–504, ISBN: 978-0-444-59576-8.
Contreras-Cornejo, H.A., Macías-Rodríguez, L., Alfaro-Cuevas, R., López-Bucio, J., 2014b. *Trichoderma* spp. improve growth of *Arabidopsis* seedlings under salt stress through enhanced root development, osmolite production, and Na^+ elimination through root exudates. Mol. Plant Microbe Interact. 27, 503–514.
Contreras-Cornejo, H.A., Macías-Rodríguez, L., Herrera-Estrella, A., López-Bucio, J., 2014c. The 4-phosphopantetheinyl transferase of *Trichoderma virens* plays a role in plant protection against *Botrytis cinerea* through volatile organic compound emission. Plant Soil 379, 261–274.
Contreras-Cornejo, H.A., Macías-Rodríguez, L., Garnica-Vergara, A., López-Bucio, J., 2015a. *Trichoderma* modulates stomatal aperture and leaf transpiration through an abscisic acid-dependent mechanism in *Arabidopsis*. J. Plant Growth Regul. 34, 425–432.
Contreras-Cornejo, H.A., López-Bucio, J.S., Méndez-Bravo, A., Macías-Rodríguez, L., Ramos-Vega, M., Guevara-García, A., López-Bucio, J., 2015b. *Trichoderma atroviride* alters *Arabidopsis* root-system architecture modulating the mitogen-activated protein kinase 6 activity through ethylene and auxin signaling pathways. Mol. Plant Microbe Interact. 28, 701–710.
De Jaeger, N., de la Providencia, I., Dupre de Boulois, H., Declerck, S., 2011. *Trichoderma harzianum* might impact phosphorus transport by arbuscular mycorrhizal fungi. FEMS Microbiol. Ecol. 77, 558–567.
Demain, A.L., Fang, A., 2000. The natural functions of secondary metabolites. Adv. Biochem. Eng. Biotechnol. 69, 1–39.
Den Herder, G., Van Isterdael, G., Beeckman, T., De Smet, I., 2010. The roots of a new green revolution. Trends Plant Sci. 15, 600–607.
Doni, F., Isahak, A., Zain, C., Ariffin, S., Mohamad, W., Yusoff, W., 2014. Formulation of *Trichoderma* sp. SL2 inoculants using different carriers for soil treatment in rice seedling growth. Springer Plus 3, 532–537.
Druzhinina, I.S., Komon-Zelazowska, M., Kredics, L., Hatvani, L., Antal, Z., Belayneh, T., Kubicek, C.P., 2008. Alternative reproductive strategies of *Hypocrea orientalis* and genetically close but clonal *Trichoderma longibrachiatum*, both capable of causing invasive mycoses of humans. Microbiology 154, 3447–3459.
Druzhinina, I.S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, C.M., Monte, E., Mukherjee, P.K., Zeilinger, S., Grigoriev, I.V., Kubicek, C.P., 2011. *Trichoderma*: the genomics of opportunistic success. Nat. Rev. Microbiol. 9, 749–759.
El-Komy, M.H., Saleh, A.A., Eranthodi, A., Molan, Y.Y., 2015. Characterization of novel *Trichoderma asperellum* isolates to select effective biocontrol agents against tomato *Fusarium* wilt. Plant Pathol. J. 31, 50–60.
Ellouze, W., Taheri, A.E., Bainard, L.D., Yang, C., Bazghaleh, N., Navarro-Borrell, A., Hanson, K., Hamel, C., 2014. Soil fungal resources in annual cropping systems and their potential for management. BioMed Res. Int., 531824.
Felten, J., Kohler, A., Morin, E., Bhalerao, R.P., Palme, K., Martin, F., Ditengou, F., Legue, V., 2009. The ectomycorrhizal fungus *Laccaria bicolor* stimulates lateral root formation in Poplar and *Arabidopsis* through auxin transport and signaling. Plant Physiol. 151, 1991–2005.
Gal-Hemed, I., Atanasova, L., Komon-Zelazowska, M., Druzhinina, I.S., Viterbo, A., Yarden, O., 2011. Marine isolates of *Trichoderma* spp. as potential halotolerant agents of biological control for arid-zone agriculture. Appl. Environ. Microbiol. 77, 5100–5109.
Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R., 2007. Applications of spray-drying in microencapsulation of food ingredients: an overview. Food Res. Int. 40, 1107–1121.
Giehl, R.F.H., von Wirén, N., 2014. Root nutrient foraging. Plant Physiol. 166, 509–517.
Gilbert, N., 2009. The disappearing nutrient. Nature 461, 716–718.
Gómez, I., Chet, I., Herrera-Estrella, A., 1997. Genetic diversity and vegetative compatibility among *Trichoderma harzianum* isolates. Mol. Gen. Genet. 256, 127–135.
Hanada, R.E., Souza, T.D., Pomella, A.W.V., Hebbar, K.P., Pereira, J.O., Ismaiel, A., Samuels, G.J., 2008. *Trichoderma martiale* sp. Nov., a new endophyte from sapwood of *Theobroma* cacao with a potential for biological control. Mycol. Res. 112, 1335–1343.
Harman, G.E., 1991. Seed treatments for biological control of plant disease. Crop Prot. 10, 166–171.
Harman, G.E., Jin, X., Stasz, T.E., Peruzzotti, G., Leopold, A.C., Taylor, A.G., 1991. Production of conidial biomass of *Trichoderma harzianum* for biological control. Biol. Control 1, 23–28.
Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2, 43–56.
Harman, G.E., 2011. Multifunctional fungal plant symbionts: new tools to enhance plant growth and productivity. New Phytol. 189, 647–649.
Hashem, A., Allah, E.F., Alqarawib, A.A., Al Huqaila, A., Egamberdieva, D., 2014. Alleviation of abiotic salt stress in *Ochradenus baccatus* (Del.) by *Trichoderma hamatum* (Bonord.) Bainier. J. Plant Int. 9, 857–868.
Hermosa, R., Viterbo, A., Chet, I., Monte, E., 2012. Plant-beneficial effects of *Trichoderma* and of its genes. Microbiology 158, 17–25.
Hilbert, M., Lars, M., Yi, D., Hofmann, J., Sharma, M., Zuccaro, A., 2012. Indole derivative production by the root endophyte *Piriformospora indica* is not required for growth promotion but for biotrophic colonization of barley roots. New Phytol. 196, 520–534.
Howell, C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis. 87, 4–10.
Hubbard, A., Lewis, C.M., Yoshida, K., Ramirez-Gonzalez, R., de Vallavieille-Pope, C., Thomas, J., Kamoun, S., Bayles, R., Uauy, C., Saunders, D., 2015. Field pathogenomics reveals the emergence of a diverse wheat yellow rust population. Genome Biol. 16 (1), http://dx.doi.org/10.1186/s13059-015-0590-8.
Hung, R., Lee, S., Bennett, J.W., 2013. *Arabidopsis thaliana* as a model system for testing the effect of *Trichoderma* volatile organic compounds. Fungal Ecol. 6, 19–26.
IFA, 2016. Fertilizer outlook 2012–2016. In: International Fertilizer Industry Association. 80th IFA Annual Conference, Doha, Qatar, p. 2012.
Jaklitsch, W.M., 2009. European species of Hypocrea part I the green-spored species. Stud. Mycol. 63, 1–91.
Jaklitsch, W.M., 2011. European species of *Hypocrea* part II: species with hyaline ascospores. Fungal Divers. 48, 1–250.

Jin, X., Harman, G.E., Taylor, A.G., 1991. Conidial biomass and desiccation tolerance of *Trichoderma harzianum* produced at different medium water potentials. Biol. Control 1, 237–243.
Jin, X., Hayes, K.C., Harman, G.E., 1992. Principles in the development of biological control systems employing *Trichoderma* species against soil-born plant pathogenic fungi. In: Lantham, G.F. (Ed.), Frontiers in Industrial Mycology. Chapman and Hall Inc., New York, pp. 174–195.
Jin, X., Custis, D., 2011. Microencapsulating aerial conidia of *Trichoderma harzianum* through spray drying at elevated temperatures. Biol. Control 56, 202–208.
Jisha, M.S., Alagawadi, A.R., 1996. Nutrient uptake and yield of sorghum (*Sorghum bicolor* (L) Moench) inoculated with phosphate solubilizing bacteria and cellulolytic fungus in a cotton stalk amended vertisol. Microbiol. Res. 151, 213–217.
John, R.P., Tyagi, R.D., Brar, S.K., Surampalli, R.Y., Prévost, D., 2011. Bio-encapsulation of microbial cells for targeted agricultural delivery. Crit. Rev. Biotechnol. 31, 211–226.
Kazan, K., 2013. Auxin and the integration of environmental signals into plant root development. Ann. Bot. 112, 1655–1665.
Kleifeld, O., Chet, I., 1992. *Trichoderma harzianum*: interaction with plants and effect on growth-response. Plant Soil 144, 267–272.
Kredics, L., Antal, Z., Dóczi, I., Manczinger, L., Kevei, F., Nagy, E., 2003. Clinical importance of the genus *Trichoderma*. A review. Acta Microbiol. Immunol. Hung. 50, 105–117.
Krouk, G., Lacombe, B., Bielach, A., Perrine-Walker, F., Malinska, K., Mounier, E., Hoyerova, K., Tillard, P., Leon, S., Ljung, K., Zazimalova, E., Benkova, E., Nacry, P., Gojon, A., 2010. Nitrate-regulated auxin transport by NRT1. 1 defines a mechanism for nutrient sensing in plants. Dev. Cell 18, 927–937.
Kubicek, C.P., Komon-Zelazowska, M., Druzhinina, I.S., 2008. Fungal genus *Hypocrea*/*Trichoderma*: from barcodes to biodiversity. J. Zhejiang Univ. Sci. B 9, 753–763.
Lace, B., Genre, A., Woo, S., Faccio, A., Lorito, M., Bonfante, P., 2015. Gate crashing arbuscular mycorrhizas: *in vivo* imaging shows the extensive colonization of both symbionts by *Trichoderma atroviride*. Environ. Microbiol. Rep. 7, 64–77.
Lahrmann, U., Ding, Y., Banhara, A., Rath, M., Hajirezaei, M.R., Döhlemann, S., von Wirén, N., Parniske, M., Zuccaro, A., 2013. Host-related metabolic cues affect colonization strategies of a root endophyte. Proc. Natl. Acad. Sci. U. S. A. 108, 7253–7258.
Lei, L., Li, Y., Wang, Q., Xu, J., Chen, Y., Yang, H., Ren, D., 2014. Activation of MKK9-MPK3/MPK6 enhances phosphate acquisition in *Arabidopsis thaliana*. New Phytol. 203, 1146–1160.
Lewis, D.R., Negi, S., Sukumar, P., Muday, G.K., 2011. Ethylene inhibits lateral root development, increases IAA transport and expression of PIN3 and PIN7 auxin efflux carriers. Development 138, 3485–3495.
López-Bucio, J.S., Dubrovsky, J.G., Raya-González, J., Ugartechea-Chirino, Y., López-Bucio, J., de Luna-Valdez, L.A., Ramos-Vega, M., León, P., Guevara-García, A., 2014. *Arabidopsis thaliana* mitogen-activated protein kinase 6 is involved in seed formation and modulation of primary and lateral root development. J. Exp. Bot. 65, 169–183.
López-Quintero, C.A., Atanasova, L., Franco-Molano, A.E., Gams, W., Komon-Zelazowska, M., Theelen, B., Müller, W.H., Boekhout, T., Druzhinina, I., 2013. DNA barcoding survey of *Trichoderma* diversity in soil and litter of the Colombian lowland Amazonian rainforest reveals *Trichoderma strigosellum* sp. Nov. and other species. Antonie Van Leeuwenhoek 10, 657–674.
Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., Abou-Hadid, A.F., El-Behairy, U.A., Sorlini, C., Cherif, A., Zocchi, G., Daffonchio, D., 2012. A drought resistance-promoting microbiome is selected by root system under desert farming. PLoS One 7, e48479.
Martínez-Medina, A., Roldán, A., Pascual, J.A., 2009. Performance of a *Trichoderma harzianum* bentonite–vermiculite formulation against *Fusarium* wilt in seedling nursery melon plants. Hortic. Sci. 44, 2025–2027.
Martínez-Medina, A., Alguacil, M., Pascual, J.A., Van Wees, S.C.M., 2014. Phytohormone profiles induced by *Trichoderma* isolates correspond with their biocontrol and plant growth-promoting activity on Melon plants. J. Chem. Ecol. 40, 804–815.
Mastouri, F., Björkman, T., Harman, G.E., 2010. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology 100, 1213–1221.
Mastouri, F., Björkman, T., Harman, G.E., 2012. *Trichoderma harzianum* enhances antioxidant defense of tomato seedlings and resistance to water deficit. Mol. Plant Microbe Interact. 25, 1264–1271.
Mathre, D., Cook, R.J., Callan, N.W., 1999. From discovery to use: traversing the world of commercializing biocontrol agents for plant disease control. Plant Dis. 83, 972–983.
Meng, X., Xu, J., He, Y., Yang, K.Y., Mordorski, Y., Liu, Y., Zhang, S., 2013. Phosphorylation of an ERF transcription Factor by *Arabidopsis* MPK3/MPK6 regulates plant defense gene induction and fungal resistance. Plant Cell 25, 1126–1142.
Ming, Q., Su, C., Zheng, C., Jia, M., Zhang, Q., Zhang, H., Rahman, K., Han, T., Qin, L., 2013. Elicitors from the endophytic fungus *Trichoderma atroviride* promote *Salvia miltiorrhiza* hairy root growth and tanshinone biosynthesis. J. Exp. Bot. 64, 5687–5694.
Molla, A.H., Haque, M.M., Haque, M.A., Ilias, G.N.M., 2012. *Trichoderma*-enriched biofertilizer enhances production and nutritional quality of tomato (*Lycopersicon esculentum* Mill) and minimizes NPK fertilizer use. Agric. Res. 1, 265–272.
Morgan, C.A., Herman, N., White, P.A., Vesey, G., 2006. Preservation of micro-organisms by drying. A review. J. Microbiol. Method 66, 183–193.
Mulaw, T.B., Kubicek, C.P., Druzhinina, I.S., 2010. The rhizosphere of *Coffea arabica* in its native highland forests of Ethiopia provides a niche for a distinguished diversity of *Trichoderma*. Fungal Divers. 2, 527–549.
Muñoz-Celaya, A.L., Ortiz-García, M., Vernon-Carter, E.J., Jauregui-Rincón, J., Galindo, E., Serrano-Carreón, L., 2012. Spray–drying microencapsulation of *Trichoderma harzianum* conidia in carbohydrate polymers matrices. Carbohydr. Polym. 88, 1141–1148.
Nelson, M.E., Powelson, M.L., 1988. Biological control of grey mold of snap beans by *Trichoderma hamatum*. Plant Dis. 72, 727–729.
O'Riordan, K., Andrews, D., Buckle, K., Conway, P., 2001. Evaluation of microbioencapsulation of a *Bifidobacterium* strain with starch as an approach to prolonging viability during storage. J. Appl. Microbiol. 91, 1059–1066.
Ortíz-Castro, R., Contreras-Cornejo, H.A., Macías-Rodríguez, L., López-Bucio, J., 2009. The role of microbial signals in plant growth and development. Plant Signal. Behav. 4, 701–712.
Ortiz-Castro, R., Díaz-Pérez, C., Martínez-Trujillo, M., del Río, R., Campos-García, J., López-Bucio, J., 2011. Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. Proc. Natl. Acad. Sci. U. S. A. 108, 7253–7258.
Ousley, M.A., Lynch, J.M., Whipps, J.M., 1994. Potential of *Trichoderma* spp. as consistent plant growth stimulators. Biol. Fertil. Soils 17, 85–90.
Owen, D., Williams, A.P., Griffith, G.W., Withers, P.J.A., 2015. Use of commercial bio-inoculants to increase agricultural production through improved phosphorus acquisition. Appl. Soil Ecol. 86, 41–54.
Papavizas, G.C., Dunn, M.T., Lewis, J.A., Beagle-Ristaino, J., 1984. Liquid fermentation technology for experimental production of biocontrol fungi. Phytopathology 74, 1171–1175.
Papavizas, G.C., 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annu. Rev. Phytopathol. 23, 23–54.
Paz, Z., Komon-Zelazowska, M., Druzhinina, I.S., Aveskamp, M.M., Shnaiderman, A., Aluma, Y., Carmeli, S., Ilan, M., Yarden, O., 2010. Diversity and potential antifungal properties of fungi associated with a Mediterranean sponge. Fungal Divers. 42, 17–26.
Pereira, J.L., Queiroz, R.M.L., Charneau, S., Felix, C.R., Ricart, C.A., Lopes da Silva, F., Stecca, A., Steindorff, C., Ulhoa, C.J., Noronha, E.F., 2014. Analysis of *Phaseolus vulgaris* response to its association with *Trichoderma harzianum* (ALL-42) in the presence or absence of the phytopathogenic fungi *Rhizoctonia solani* and *Fusarium solani*. PLoS One 9, e98234.
Ramírez-Chávez, E., López-Bucio, J., Herrera-Estrella, L., Molina-Torres, J., 2004. Alkamides isolated from plants promote growth and alter root development in *Arabidopsis*. Plant Physiol. 134, 1058–1068.
Rathore, S., Mahendrakumar, D., Liew, C.V., Chan, L.W., Heng, P.W., 2013. Microencapsulation of microbial cells. J. Food Eng. 116, 369–381.
Rawat, R., Tewari, L., 2011. Effect of abiotic stress on phosphate solubilization by biocontrol fungus *Trichoderma* sp. Curr. Microbiol. 62, 1521–1526.
Rawat, L., Singh, Y., Shukla, N., Kumar, J., 2013. Salinity tolerant *Trichoderma harzianum* reinforces NaCl tolerance and reduces population dynamics of *Fusarium oxysporum* f. sp. Cicero in chickpea (*Cicer arietinum* L.) under salt stress conditions. Arch. Phytopathol. Plant Prot. 46, 1442–1467.
Reino, J.L., Guerriero, R.F., Hernandez-Gala, R., Collado, I.G., 2008. Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochem. Rev. 7, 89–123.
Ross, R.P., Desmond, C., Fitzgerald, G.F., Stanton, C., 2005. Overcoming the technological hurdles in the development of probiotic foods. J. Appl. Microbiol. 98, 1410–1417.
Rossmann, A.Y., Samuels, G.J., Rogerson, C.T., Lowen, R., 1999. Genera of *Bionectriaceae*, *Hypocreaceae* and *Nectriaceae* (*Hypocreales*, *Ascomycetes*). Stud. Mycol. 42, 1–248.
Rousseau, A., Benhamou, N., Chet, I., Piche, Y., 1996. Mycoparasitism of the extramatrical phase of *Glomus intraradices* by *Trichoderma harzianum*. Phytopathology 86, 434–443.
Rubio, M.B., Dominguez, S., Monte, E., Hermosa, R., 2012. Comparative study of Trichoderma gene expression in interactions with tomato plants using high-density oligonucleotide microarrays. Microbiology 158, 119–128.
Rubio, M.B., Quijada, N.M., Pérez, E., Domínguez, S., Monte, E., Hermosa, R., 2014. Identifying beneficial qualities of *Trichoderma parareesei* for plants. Appl. Environ. Microbiol. 80, 1864–1873.
Rudresh, D.L., Shivaprakash, M.K., Prasad, R.D., 2005a. Tricalcium phosphate solubilizing abilities of *Trichoderma* spp. in relation to P uptake and growth yield parameters of chickpea (*Cicer arietinum* L.). Can. J. Microbiol. 51, 217–226.
Rudresh, D.L., Shivaprakash, M.K., Prasad, R.D., 2005b. Effect of combined application of *Rhizobium*, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer arietinum* L.). Appl. Soil Ecol., 139–146.
Ruiz-Herrera, L.F., Shane, M., López-Bucio, J., 2015. Nutritional regulation of root development. Wiley Interdiscip. Rev. Dev. Biol. 4, 431–443.
Samolski, I., Rincón, A.M., Pinzón, L.M., Viterbo, A., Monte, E., 2012. The *qid74* gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization. Microbiology 158, 129–138.
Samuels, G.J., 1996. *Trichoderma*: a review of biology and systematics of the genus. Mycol. Res. 100, 923–935.
Shoresh, M., Harman, G.E., 2008. The molecular basis of shoot responses of maize 517 seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach. Plant Physiol. 147, 2147–2163.

Shoresh, M., Harman, G.E., Mastouri, F., 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. Annu. Rev. Phytopathol. 48, 1–23.
Shukla, N., Awasthi, R.P., Rawat, L., Kumar, J., 2012. Biochemical and physiological responses of rice (*Oryza sativa* L.) as influenced by *Trichoderma harzianum* under drought stress. Plant Physiol. Biochem. 54, 78–88.
Simon, S., Kube, M., Baster, P., Robert, S., Dobrev, P.I., Friml, J., Petrasek, J., Zazımalova, E., 2013. Defining the selectivity of processes along the auxin response chain: a study using auxin analogues. New Phytol. 200, 1034–1048.
Sofo, A., Milella, L., Tataranni, G., 2010. Effects of *Trichoderma harzianum* strain T-22 on the growth of two *Prunus* rootstocks during the rooting phase. J. Hortic. Sci. Biotechnol. 85, 497–502.
Sofo, A., Scopa, A., Manfra, M., De Nisco, M., Tenore, G., Troisi, J., Di Fiori, R., Ettore, N., 2011. *Trichoderma harzianum* strain T-22 induces changes in phytohormone levels in cherry rootstocks (*Prunus cerasus* × *P canescens*). Plant Growth Regul. 65, 421–425.
Stoppacher, N., Kluger, B., Zeilinger, S., Krska, R., Schuhmacher, R., 2010. Identification and profiling of volatile metabolites of the biocontrol fungus Trichoderma atroviride by HS–SPME–GC–MS. J. Microbiol. Method 81, 187–193.
Studholme, D.J., Harris, B., LeCocq, K., Winsbury, R., Perer, A.V., Ryder, L., Ward, J.L., Beale, M.H., Thornton, C.R., Grant, M., 2013. Investigating the beneficial traits of *Trichoderma hamatum* GD 12 for sustainable agriculture-insights from genomics. Front. Plant Sci., http://dx.doi.org/10.3389/fpls.2013.00258.
Tucci, M., Ruocco, M., De Masi, L., De Palma, M., Lorito, M., 2011. The beneficial effect of *Trichoderma* spp. on tomato is modulated by the plant genotype. Mol. Plant Pathol. 12, 341–354.
Vargas, W.A., Mandawe, J.C., Kenerley, C.M., 2009. Plant-derived sucrose is a key element in the symbiotic association between *Trichoderma virens* and maize plants. Plant Physiol. 151, 792–808.
Vargas, W.A., Crutcher, F.K., Kenerley, C.M., 2011. Functional characterization of a plant-like sucrose transporter from the beneficial fungus *Trichoderma virens*. Regulation of the symbiotic association with plants by sucrose metabolism inside the fungal cells. New Phytol. 189, 777–789.
Verma, M., Brar, S., Tyagi, R., Surampalli, R., Valero, J., 2007. Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. Biochem. Eng. J. 37, 1–20.
Vinale, F., Sivasithamparam, K., Ghisalberti, E., Marra, R., Barbetti, M., Li, H., Woo, S., Lorito, M., 2008. A novel role for *Trichoderma* secondary metabolites in the interactions with plants. Physiol. Mol. Plant Pathol. 72, 80–86.
Vinale, F., Manganiello, G., Nigro, M., Mazzei, P., Piccolo, A., Pascale, A., Ruocco, M., Marra, R., Lombardi, N., Lanzuise, S., Varlese, R., Cavallo, P., Lorito, M., Woo, S., 2014. A novel fungal metabolite with beneficial properties for agricultural applications. Molecules 19, 9760–9772.
Whipps, J.M., 1997. Developments in the biological control of soil-borne plant pathogens. Adv. Bot. Res. 26, 1–34.
Xiang, W., Zhao, L., Xu, X., Qin, Y., Yu, G., 2012. Mutual information flow between beneficial microorganisms and the roots of host plants determined the bio-functions of biofertilizers. Am. J. Plant Sci. 3, 1115–1120.
Xie, B.B., Qin, Q.L., Shi, M., Chen, L.L., Shu, Y.L., Luo, Y., Wang, X.W., Rong, J.C., Gong, Z.T., Li, D., Sun, C.Y., Liu, G.M., Dong, X.W., Pang, X.H., Huang, F., Liu, W., Chen, X.L., Zhou, B.C., Zhang, Y.Z., Song, X.Y., 2014. Comparative genomics provide insights into evolution of *Trichoderma* nutrition style. Genome Biol. Evol. 6, 379–390.
Xu, J., Zhang, S., 2015. Mitogen-activated protein kinase cascades in signaling plant growth and development. Trends Plant Sci. 20, 56–64.
Yedidia, I., Srivastva, A.K., Kapulnik, Y., Chet, I., 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant Soil 235, 235–242.
Yobo, K.S., Laing, M.D., Hunter, C.H., 2009. Effects of single and dual applications of selected *Trichoderma* and *Bacillus* isolates on performance of dry bean seedlings grown in composted pine bark growth medium under shadehouse conditions. J. Plant Nutr. 32, 1271–1289.
Yobo, K.S., Laing, M.D., Hunter, C.H., 2011. Effects of single and combined inoculations of selected *Trichoderma* and *Bacillus* isolates on growth of dry bean and biological control of *Rhizoctonia solani* damping-off. Afr. J. Biotechnol. 10, 8746–8756.
Zhang, C.L., Liu, S.P., Lin, F., Kubicek, C.P., Druzhinina, I.S., 2007. *Trichoderma* taxi sp. nov., an endophytic fungus from Chinese yew *Taxus mairei*. FEMS Microbiol. Lett. 270, 90–96.
Zhao, L., Wang, F., Zhang, Y., Zhang, J., 2014. Involvement of *Trichoderma asperellum* strain T6 in regulating iron acquisition in plants. J. Basic Microbiol. 54, 115–124.
Zörb, C., Geilfus, C.M., Mühling, K.H., Ludwig-Müller, J., 2013. The influence of salt stress on ABA and auxin concentrations in two maize cultivars differing in salt resistance. J. Plant Physiol. 170, 220–234.