



Review Paper

A review on interactions of *Trichoderma* with Plant and Pathogens

Rai Dinesh and Mehra Prateeksha

Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar–263145, Uttarakhand, INDIA

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Abstract

Trichoderma spp. are the most successful bio-fungicides used in today's agriculture with more than 60 % of the registered bio-fungicides world-wide being *Trichoderma* based. Induction of plant defense and mycoparasitism (killing of one fungus by another) are considered to be the most important mechanisms of *Trichoderma* mediated biological control. Several strategies have been applied to identify the main genes and compounds involved in this complex, three-way cross-talk between the fungal antagonist, the plant, and microbial pathogens. Understanding these mechanisms at the molecular level would help in developing strains with superior biocontrol properties. In this article, we review our current understanding of the genetics of interactions of *Trichoderma* with plants and plant pathogens.

Keywords: *Trichoderma*, interaction, plant, pathogen.

Introduction

Trichoderma spp. are very useful filamentous fungi. By producing beneficial effects on crops, they have naturally sustained the agricultural yields that have supported the human population over the millennia. Together with other beneficial microbes, they help maintain the general disease suppressiveness and fertility of soils, and aid in the maturation of compost for natural fertilizer production¹. *Trichoderma* spp. are ubiquitous and often predominant components of the mycoflora in native and agricultural soils throughout all climatic zones. They colonize aboveground and belowground plant organs and grow between living cells (endophytes), and they appear in plant litter, soil organic matter (saprophytes), and mammalian tissues (human pathogens). However, the ability of these fungi to sense, invade, and destroy other fungi has been the major driving force behind their commercial success as biopesticides. These fungi not only protect plants by killing other fungi and certain nematodes but induce resistance against plant pathogens, impart abiotic stress tolerance, improve plant growth and vigor, solubilize plant nutrients, and bioremediate heavy metals and environmental pollutants. In addition, this genus comprises fungi that produce secondary metabolites of clinical significance and enzymes with widespread industrial application. Better understanding of how *Trichoderma* evolved to interact with other fungi and with plants will improve and expand their applications. The ability to attack other fungi, most importantly soilborne plant dominated the interest in *Trichoderma* for many years. Recent years have witnessed a wave of interest in plant disease resistance [induced systemic resistance (ISR); to some extent, systemic acquired resistance (SAR)] induced by the *Trichoderma*-root symbiosis. These plant-centered mechanisms have rivaled mycoparasitism as an explanation for how *Trichoderma* controls plant diseases. The genome sequencing of *Trichoderma* species has stimulated the

development of systems biological approaches, initiated and enhanced whole-genome expression studies, and provided unique data for phylogenetic and bioinformatic analyses toward understanding the roles of these opportunists in ecosystems². At present the, genome sequences of seven species: *Trichoderma reesei*, *Trichoderma virens*, *Trichoderma atroviride*, *Trichoderma harzianum*, *Trichoderma asperellum*, *Trichoderma longibrachiatum*, and *Trichoderma citrinoviride* are available³. The genome of *Trichoderma* spp. has been extensively investigated and has proven to contain many useful genes, along with the ability to produce a great variety of expression patterns, which allows these fungi to adapt to many different environments (soil, water, dead tissues, inside the plant, etc.). Several laboratories have recently started or planned to use proteomic and functional genomic analysis in the attempt to obtain an overall picture of the changes that occur in the *Trichoderma*, plant, and pathogen expressomes when they "talk" to each other, especially when an increase in disease resistance is generated.

Trichoderma spp. are the most successful bio-fungicides used in today's agriculture with more than 60 % of the registered bio-fungicides world-wide being *Trichoderma*-based⁴. In India alone, about 250 products are available for field applications⁵. Despite this remarkable success, the share of bio-fungicides is only a fraction of the fungicides market, dominated by synthetic chemicals. The major limitations of microbe-based fungicides are their restricted efficacy and their inconsistency under field conditions. The origin of these difficulties is that microbes are slow to act, compared to chemicals, and are influenced by environmental factors. Here, "genetic intervention" to design strains that are more effective than the native ones might prove useful. This goal could be attained by gaining knowledge on the molecular mechanisms of interactions of these organisms with other biotic and abiotic factors. We summarize here the recent

findings on the genetics of interactions of *Trichoderma* with plants and pathogens.

Trichoderma-plant interactions

Many *Trichoderma* spp. grow in the rhizosphere and are capable of penetrating and internally colonizing plant roots¹. This opportunistic/facultative symbiosis is driven by the ability of *Trichoderma* to derive sucrose or other nutrients from plants, in return for boosting plant immunity against invading pathogens. The presence of *Trichoderma* in the rhizosphere evokes a coordinated transcriptomic, proteomic and metabolomic response in the plant⁶. This reprogramming of the plant is often beneficial, improving growth, yield and resistance to pathogens. The combined ability to attack soilborne pathogens while priming plant defenses, however, is what promotes *Trichoderma* as such a promising partner for sustainable management of plant diseases.

Root colonization

Trichoderma spp. can colonize plant roots, both externally and internally. *Trichoderma* interaction with plant roots results in controlled activation of carbohydrate metabolic processes as well as enhancement of photosynthesis, providing the growing plant with more energy and carbon source for their growth. Other growth signals may also be induced. Stress and defense related pathways are also induced, probably involving ethylene signal transduction. Induction of cell wall metabolism may serve to strengthen cell barriers, adding to the resistance of the plants. The primary step in the *Trichoderma*-plant interaction is rather poorly understood compared to those that follow, i.e., attachment, penetration and internal colonization of plant roots. *Trichoderma* spp. produce and modulate hormonal signals in order to facilitate the colonization of roots. The fungus produces auxins that promote root growth which, in turn, facilitates colonization by increasing the available surface area⁷. *Trichoderma* deploys small secreted cysteine-rich hydrophobin-like proteins to facilitate anchoring/ attachment. Two such proteins have been found to facilitate attachment to the roots-TasHyd1 from *T. asperellum* and Qid74 of *T. harzianum*^{8,9}. Qid74 has an important role in lateral root growth and hair formation and elongation. Modification of root architecture increases the total absorptive surface, facilitating nutrient uptake and translocation of nutrients in the shoots, and resulting in increased plant biomass through an efficient use of mineral elements. *Trichoderma* spp. secretes expansin-like proteins with cellulose binding modules and endopolygalacturonase to facilitate root penetration. Once inside the roots, these fungi can grow intercellularly, albeit limited to epidermal layer and outer cortex. Initial suppression of plant defense may facilitate root invasion.

Induced defense

Trichoderma species are beneficial opportunistic fungi which, apart from being very efficient mycoparasites, can interact with

roots of diverse plant species leading to induced systemic resistance (ISR) responses to a wide spectrum of pathogens and adverse environmental conditions¹⁰. *T. hamatum* 382 systemically induced about 40 genes in tomato plants with functions associated with biotic or abiotic stress, as well as RNA, DNA and protein metabolism^{11,12}. A total of 205 differentially expressed proteins, over both roots and shoots, were identified in maize plants inoculated by *T. harzianum* T22⁶.

Plants respond immediately to *Trichoderma* invasion by rapid ion fluxes and an oxidative burst, followed by deposition of callose and synthesis of polyphenols. Subsequent events involve salicylate (SA) and jasmonate/ ethylene (JA/ET)-signaling, which results in the entire plant acquiring varying degrees of tolerance to pathogen invasion¹⁰. This response has, most frequently, been described as JA/ET-mediated induced systemic resistance (ISR) and resembles the response triggered by plant growth-promoting rhizobacteria (PGPR). Recent findings, however, indicate that at higher inoculum doses *Trichoderma* can trigger a SA mediated systemic acquired resistance (SAR) response, similar to that invoked by necrotrophic pathogens.

Xylanase and peptaibols (peptaibiotics with high content of alpha amino isobutyric acid) like alamethicin and trichovirin II which are produced by *Trichoderma* spp. were shown to elicit an immune response in plants¹³. The best characterized elicitor produced by *Trichoderma* spp. is Sm1/Epl1, an abundantly secreted, small cysteine-rich hydrophobin-like protein of the cerato-platanin (CP) family^{14,15}. The monomeric form of Sm1 is in a glycosylated state which is essential for elicitation properties. It was suggested that the monomeric form in the non-glycosylated state is susceptible to oxidative-driven dimerization in plants rendering Sm1 inactive as inducer of ISR. Recently, the 3-D structure of the *Ceratocystis platani* cerato-platanin has been resolved and the carbohydrate residue (an oligomer of N-acetyl glucosamine) that binds to it has been identified. Since the CP protein family is highly conserved, its structure and carbohydrate-binding properties may suggest a mechanism for the elicitation properties of Sm1.

The Endophytic *Trichoderma*

Recent reports suggest that some *Trichoderma* spp. are not restricted to outer root tissues, but can also live in the plant as "true" endophytes¹³. Interestingly, most of the endophytic *Trichoderma* discovered are "new" species (e.g., *T. stromaticum*, *T. amazonicum*, *T. evansii*, *T. martiale*, *T. taxi* and *T. theobromicola*), different from those routinely isolated from soil/ rhizosphere and a phylogenetic analysis revealed that these species are of recent evolutionary origin. The endophytic *Trichoderma* species are reported to induce transcriptomic changes in plants and some are known to protect plants from diseases and abiotic stresses. Some of these endophytes preferentially colonize the surface of glandular trichomes and form appressoria-like structures¹⁶.

Interactions with plant pathogens

Mycoparasitism is apparently an ancestral trait of *Trichoderma*¹⁷. The ability of *Trichoderma* to parasitize and kill other fungi has been the major driving force behind the commercial success of these fungi as biofungicides. In addition, some *Trichoderma* spp. can kill nematodes and hence have the potential for applications as bio-nematicides. A typical mycoparasitic interaction involves sensing of the host/ prey fungus, attraction, attachment, coiling around and lysis brought about by hydrolytic enzymes, in many cases, in conjunction with secondary metabolites.

Environmental signaling plays an important role in cellular organisms. Understanding of the mechanisms of cell signaling in *Trichoderma* is limited compared to “model” fungi like *Magnaporthe grisea* and *Neurospora crassa*, but there has been significant progress based on genetic approaches. The seven transmembrane G protein-coupled receptor Gpr1 is involved in sensing the fungal prey: silencing of the *gpr1* gene in *T. atroviride* rendered the mycoparasite unable to respond to the presence of the host fungus¹⁸. Binding of a ligand to such receptors leads to downstream signaling events via activation of G-protein cascades. Like most other filamentous fungi, *Trichoderma* spp. have three MAPK cascades comprising MAPKKK, MAPKK and MAPK¹⁹ and MAPK pathways may act in mycoparasitism and biocontrol.

Attachment to host fungi

Attachment to and attack of host fungi by mycoparasitic *Trichoderma* is accompanied by the formation of appressoria- or papillae-like structures and/or coiling around host hyphae¹³. The genetics underlying attachment of the mycoparasite to the host fungus are not well understood, although hydrophobins are possibly involved. Though experimental evidence is lacking, indirect support for the involvement of hydrophobins comes from the finding that *T. virens* mutants in the transcriptional regulator of secondary metabolism and morphogenesis *Vel1*, which have decreased hydrophobin expression, were defective in both hydrophobicity and mycoparasitism²⁰.

Killing the host

Hydrolytic enzymes and antibiotics are among the most important members of the chemical arsenals deployed by *Trichoderma* to kill other fungi. Not surprisingly, the genomes of the mycoparasitic *Trichoderma* spp. are rich in genes encoding enzymes like chitinases and glucanases, and those for secondary metabolism like NRPSs¹⁷. Glucanases are another group of cell wall-lytic enzymes with roles in mycoparasitism/ biocontrol. Deletion of *tvbgn3* (b-1,6-glucanase- encoding) reduced the mycoparasitic and biocontrol potential of *T. virens* against *P. ultimum*²¹. In addition to chitinases and glucanases, proteases like Prb1/Sp1 are induced during mycoparasitism and play definitive roles in biocontrol²².

In contrast to studies on hyphal parasitism, very little research has been done on the molecular mechanisms of parasitism of resting structures.

Trichoderma spp. are prolific producers of secondary metabolites and the genomes of the mycoparasitic *Trichoderma* spp. are especially enriched in genes for secondary metabolism²³. Roles of antimicrobial secondary metabolites such as gliotoxin and gliovirin in suppression of *R. solani* and *P. ultimum* have been suggested, although contradictory reports exist²¹. Certain species like *T. atroviride* produce the volatile metabolite which plays an important role in *Trichoderma*–plant and *Trichoderma*–fungal interactions.

Conclusion

Genetic basis of interaction of *Trichoderma* with other (notably fungi and plants), an in depth understanding of the mechanisms is lacking. The absence of high throughput studies in these organisms has been due to the lack of whole genome sequences. However, this scenario is now expected to change with the availability of numerous *Trichoderma* genome sequences would facilitate interchange of research among the different species of the genus. The genome of *Trichoderma* spp. has been extensively investigated has proven to contain many useful genes, along with the ability to produce a great variety of expression patterns, which allows these fungi to adapt to many different environments (soil, water, dead tissues, inside the plant, etc.). Finally, understanding the mechanism of interaction between *Trichoderma* spp. and the plant has provided for the first time the opportunity to genetically increase the ability of a *Trichoderma* strain for the management plant diseases.

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