Enhancement of plant disease resistance by the biocontrol agent *Trichoderma*

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Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection. Our main research interest focuses on a novel approach for induction of local and induced systemic resistance (ISR) towards plant pathogens, based on a "mycorrhiza-like" saprophytic fungus, *Trichoderma*, usually used as a biocontrol agent. The main objectives of our research are on one hand to isolate molecules secreted by the fungus involved in different stages of the process, from the root colonization to the recognition signaling between the plant and the fungus, and on the other to monitor changes in the plant due to the presence of the colonizing fungus.

Trichoderma spp. are fungi that are present in nearly all agricultural soils and in other environments such as decaying wood. The antifungal abilities of these beneficial microbes have been known since the 1930s, and there have been extensive efforts to use them for plant disease control since then. These fungi grow tropically towards hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target fungi by the secretion of different lytic enzymes. This process (mycoparasitism) limits growth and activity of plant pathogenic fungi.

Some *Trichoderma* rhizosphere-competent strains colonize also entire root surfaces with morphological features reminiscent of those seen during mycoparasitism. Penetration of the root tissue is usually limited to the first or second layers of cells and only in the intercellular spaces. *Trichoderma* strains capable of establishing such interaction induce metabolic changes in plants that increase resistance to a wide range of plant-pathogenic microorganisms and viruses (Harman et al, 2004). This response seems to be broadly effective for many plants, which indicates that there is little or no plant specificity. At least three classes of substance that elicit plant defense responses have been identified. These elicitors include peptides, proteins and low-molecular weight compounds (Harman et al., 2004, Viterbo et al., 2004). The systemic response in plants occurs through the JA/ethylene signaling pathway in a similar manner to the rhizobacteria-induced systemic resistance (Shoresh et al., 2005). Several studies have shown that root colonization by

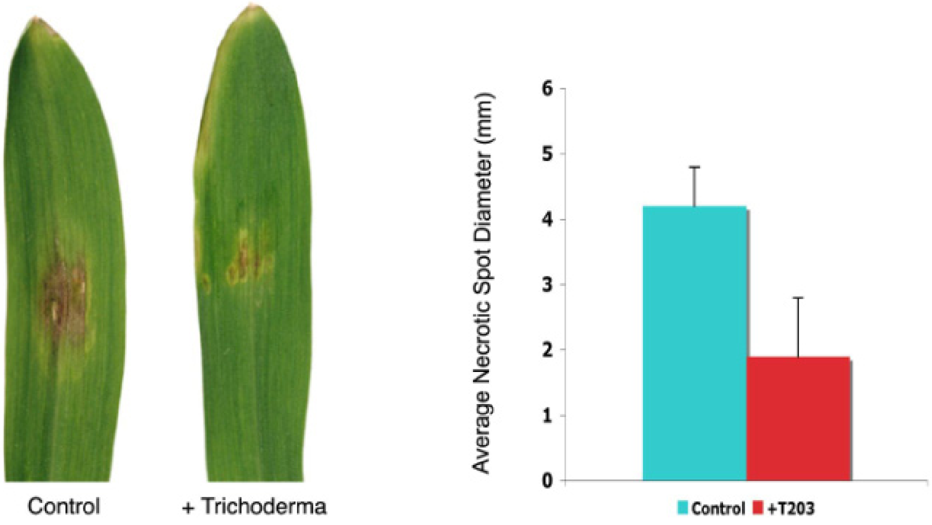

Fig 1. *Induced resistance towards the leaf pathogen Cochliobolus heterostrophus in maize. Seedling roots were infected with germinated Trichoderma spores (10^5/ml) 48h prior to pathogen leaf infection (800 spores). The symptoms were recorded 72h after infection.*

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Trichoderma strains results in massive changes in the plant genome and metabolome. Changes in plant metabolism lead to the accumulation of antimicrobial compounds. In cucumber, root colonization by *T. asperellum* strain T-203 causes an increase in phenolic glucoside levels in leaves, which are strongly inhibitory to a range of bacteria and fungi spp. (Yedidia et al., 2003). The protection afforded by the biocontrol agent is associated with the accumulation of mRNA of two defense genes: the phenylpropanoid pathway gene phenylalanine ammonia lyase (*PAL*) and the lipoxygenase pathway gene hydroxyperoxide lyase (*HPL*) (Yedidia et al., 2003). In *Trichoderma* treated cucumber seedlings upon pathogen challenge, increased levels of other defense-related plant enzymes, such as peroxidases, chitinases, and β-1, 3-glucanases has been recorded (Shoresh et al., 2005). This potentiation in the gene expression enables *Trichoderma* treated plants to be more resistant to subsequent pathogen infection. The MAPK signal transduction pathways, both of the plant and *Trichoderma*, are important for the induction of systemic resistance (Viterbo et al., 2005 and Shoresh et al., unpublished). The signals remain to be identified, but it seems that fungal MAPK pathways may transduce plant signals, telling the fungus to release factors that are in turn detected by the plant. Plants recognize microorganisms by a variety of mechanisms, and the *Trichoderma*-plant interaction might be similar to plant-pathogen interactions that are governed by pathogen-derived *avr* genes and corresponding resistance genes in the plant host. Plant defense responses can be induced, for example, by oligosaccharide elicitors, both of plant origin (pectin and cellulose or hemicellulose oligomers) and of fungal origin (chitin or glucan oligomers). The signals of plant origin are produced by cell-wall-degrading hydrolases of fungi or bacteria, while those of fungal origin are produced by plant pathogenesis-related proteins (chitinases and glucanases) or fungal enzymes secreted during mycelial growth. *Trichoderma* species secrete a variety of hydrolytic enzymes, which could release oligosaccharide signals inciting plant defense.

In order to identify molecular factors directly involved in the *Trichoderma*-plant interaction, we are conducting PCR differential screening of *T. asperellum* genes and proteomic analyses of *Trichoderma* cell wall proteins induced during root colonization. Among several clones we identified a Class I hydrophobin named *Hyd1*. Genetic functional analysis demonstrates that this protein has a role in plant root colonization and infection. In several biotrophic fungi hydrophobins have been postulated to participate in interactions associated with infection of either plant or animal hosts. Hydrophobins mediate attachment and generate signals for differentiation of structures required for penetration of the host tissue. Hydrophobins have also been suggested to act as a stealth factor protecting the invading fungus from rejection by the plant or animal hosts. Assembled hydrophobins are extremely resistant toward chemical and enzymatic treatment and can thereby protect emergent structures from adverse environmental conditions. *HYD1* might also protect the growing hyphae of *Trichoderma* from locally synthesized plant defense compounds during the first stages of plant interaction allowing this opportunistic, non-pathogenic fungus to colonize the intercellular spaces of the plant root.

By understanding the mode of action by *Trichoderma* spp. we should be able to manipulate the plant, the fungal agent and their interactions to achieve more effective plant resistance to various biotic and abiotic stresses.

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Acknowledgements

This work is supported by a United States-Israel Binational Agricultural Research and Development Fund (US-3507-04); The Dr. Alexander and Myrna Strelinger Endowment Fund.