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## PROTEOMIC STUDY OF IN VIVO THREE-WAY INTERACTIONS BETWEEN PLANT, PATHOGENS AND ANTAGONISTIC FUNGI

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Multiple factors are involved in the complex interactions between plants and microorganisms. Several studies have been carried out by using *in vitro* systems in which only two players (plant with pathogen or biocontrol agent; biocontrol agent with pathogen) have been considered. The aim of this work was to investigate the main factors that regulate the interaction between plants and fungal pathogens when biocontrol agents are involved.

*Trichoderma* spp. are among the best studied antagonistic fungi for use in agriculture as biological control agents. Their ability to limit the attack of pathogens and simultaneously increase plant growth and development has supported their application as biopesticides.

In order to isolate and characterise the main molecular factors produced by each component (plant, pathogen and biocontrol agent) during the interaction, a proteomic approach was used. 2D maps were obtained from co-cultures of bean plants, fungal pathogens (*Rhizoctonia solani, Botrytis cinerea*) and the antagonistic fungus *Trichoderma atroviride* strain P1, by using immobilised pH gradient (IPG) strips for isoelectrofocusing (IEF) followed by SDS-PAGE. Software-based analysis revealed many differential proteins produced by each organism during dual and triple interactions. The most interesting spots were further characterised by mass spectrometry (MALDI-TOF MS) and *in silico* analysis via peptide mass fingerprinting (PMF). Pathogenesis- and disease resistance-related proteins have been found to accumulate in the plant during its interaction with *R. solani* and *T. atroviride*. This finding is related to the ability of *Trichoderma* to induce plant defence mechanisms possibly by promoting the expression of resistance genes. Moreover, in the proteome of the antagonist we found a variety of new differentially expressed proteins involved in biocontrol, such as ABC transporters, hydrophobins, cyclophilins, etc. This approach will help to identify the factors involved in some of the most complex plant-microbe.