Proteomic Analyses of Susceptible and Resistant Cotton Roots to Meloidogyne incognita

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In the last decade the world cotton consumption comes growing considerably. However, the production of this culture in Brazil is affected by great losses caused mainly by pests. Among them stands out the root-knot nematode, Meloidogyne incognita. Currently, there is a global effort to control the progress of this plant pathogen. A guite promising approach to achieve this target relies on the proteomic technology. With the objective to identify proteins related to cotton resistance mechanism, a comparative studies using susceptible and resistant cotton roots from a time course (5 and 15 days after infection) have been accomplished using two-dimensional electrophoresis (2DE). The experiments were conducted in green house using plants with two months age and 30cm height. As cotton is a recalcitrant plant, three methods for the protein sample preparation have been tested. The best result has been obtained extracting the proteins with buffer (40mM Tris-HCl pH 7.0, 250mM sucrose, 1% Triton X-100, 10mM EDTA, PMSF and DTT 1mM) and precipitating them with 10% trichloroacetic acid in acetone at -20°C, overnight. The proteins were resolubilized in buffer containing 7M urea, 2M tiourea, 4% CHAPS, 2% IPG Buffer and 0.3% DTT for 2h at room temperature and submitted to 2DE. Preliminary results have been demonstrated the presence of several proteins with different expression level for the susceptible and resistant plants with and without infection. The comparison between the non infected control plants allowed the identification of six spots with higher expression in relation to the susceptible genotype and three spots present only in the resistant genotype. Five days after infection with 20,000 second stage juvenile nematodes, two spots with increased expression and two new spots, found exclusively in the resistant genotype, were visualized. Fifteen days after infection were visualized four new spots present only in the resistant genotype and two spots with increased expression in relation to susceptible genotype. These spots were excised from 2D gels, digested with tripsin and are being analyzed using a MALDI-ToF/ToF mass spectrometer. The results demonstrated the potential of proteomics in the search of proteins and/or genes of biotechnological interest for the resistance to nematodes.