

Comparative analysis of three different development phases of *Meloidogyne incognita* by two-dimensional electrophoresis

Casado-Filho, E. ¹; Rocha, T. L. ²; Mehta, A. ²; Evaristo, R.G.S. ²; Noronha, E. F. ¹; Grossi-de-Sá, M.F. ^{1,2}; Murad, A.M. ¹; Franco, O.L. ¹

¹ Centro de Análises Proteômicas e Bioquímicas; Programa de Pós-Graduação em Ciências Genômicas e Biotecnologia, Universidade Católica de Brasília; Brasília - DF - Brazil;

² Embrapa Recursos Genéticos e Biotecnologia; Brasília-DF, Brazil.

Corresponding author : ocfranco@pos.ucb.br

Phytopathogenic nematodes are able to infest several important crops, leading to worldwide annual losses around US\$125 billions. These parasites invade roots at elongation region migrating through cells until vascular tissues, causing cell damage and also stopping cell division. Different strategies have been used for nematode control but, in fact, they are inefficient and also extremely toxic for environment. Aiming the development of novel treatments towards *M. incognita*, proteins extracted from eggs, second stage juveniles (J₂) and females were analyzed by proteomic techniques as 2D gels. Samples were macerated, resuspended in Na₂PO₄ (50mM) buffer pH 7.2 containing NaCl (30 mM), PMSF (2 mM) and DTT (10mM) and centrifuged at 14.000rpm. Supernatant was precipitated using 75% trichloroacetic acid, quantified by Bradford method and applied into slab mini 15% SDS-PAGE. This first molecular mass analysis demonstrated a broad range of proteins from 10 to 200 kDa. Two-dimensional electrophoresis analyses were carried out in triplicate using 18cm immobilized 3-10pH range strips from GE HealthCare. Second dimension were done using 12% SDS-PAGE into an ISO-Dalstix system. After electrophoresis, resultant gels were silver stained. Results showed remarkable differences in proteomical pattern between different nematode development stages. Egg sample showed neutral proteins; J₂ has broad range pH while females showed acid proteins. Differences of molecular weight among the three samples were beside of the point question. The identification of different proteins from all these stages will be crucial to understand the plant-nematode interaction at molecular level, including infection and survival. This data might be used for development of novel specific and effective nematode control methods.

Financial Support: UCB; CNPq