Quarantine root-knot nematodes : specific determination and study of proteins implied in virulence/avirulence

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Quarantine root-knot nematodes : specific determination and study of proteins implied in virulence/avirulence.

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Breeders and official phytosanitary services need to identify root-knot nematode as *Meloidogyne chitwoodi* and *M. fallax* with reliable methods. A test to discriminate quarantine nematodes could be now effective and the study of virulence/avirulence of those nematodes allows to identify proteins implied in such process.

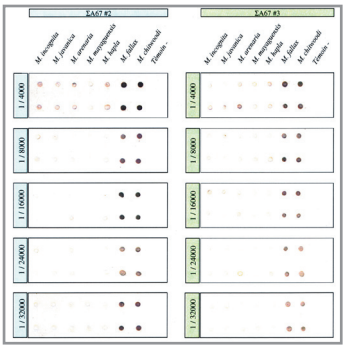
Serological identification of *M. chitwoodi* and *M. fallax*

Bidimensional electrophoresis (E2D) have been realised upon 10 species of *Meloidogyne.*

Studied species : *M. incognita, M. javanica, M. arenaria, M. mayaguensis, M. exigua, M. arabica, M. naasi, M. hapla race B*
Including quarantine species : *M. chitwoodi, M. fallax*

Two major proteins, Mcf-A67 and Mcf-B66, have been observed as differentials between quarantine nematodes and others. They have been isolated and sequenced by HPLC after endoprotease digestion.

A part of Mcf-A67 sequence, composed of 20 amino acids, shows homology with fatty acid biding proteins (FABPs) of *Caenorhabditis elegans.* Mcf-A67 was used for serological diagnostic.



Dot blots on individual females with ΣA67 serums.

The sensibility and the specificity of the immuno reaction were tested. For the detection, a minimum of 8 ng of protein is necessary. Dot blots upon individual females give very good results especially at high dilution level.

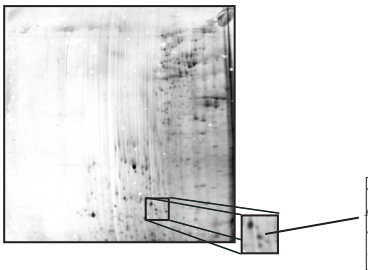
Proteome differential analysis of virulent/avirulent lines of *M. chitwoodi*

Proteome of 4 lines of *M. chitwoodi* were obtained from the same population virulent related to RMc1 resistance gene of *Solanum bulbocastanum.*

Virulence (+) and avirulence (-) of the 4 lines (V#) of M. chitwoodi on S. bulbocastanum clones.

	SB4	93-57-5	93-60-2	SB22
V1	+	+	+	-
V3	+	+	+	+
V5	+	+	-	-
V6	+	+	+	-

49 E2D gels were analysed with Kepler software. From 3 pH ranges, 3-5, 5-8, 8-10, 36 proteins were localised as specifically present or absent in one line.



Spot specifically present in V1 line in a 3-5 pH range E2D gel.

By pI and molecular weight estimation, a first investigation in protein databases (NCBI, Swissprot and TrEMBL) was done. The results showed that most part of candidate proteins could match with i.e. cuticle or secreted proteins.

Possible family and function of candidate proteins putatively implied in virulence/ avirulence process.

Proteins family	Fonction
14-3-3	Protein-protein interaction (ethylen pathway)
Oesophageal secretion	T seleno-transferin family
Cuticle collagen	Extra-cellular structure proteins
VAP-1	Pathogen related-1 like proteins
Proteinase serin	Peptidases
Hypoderm secretion	Anti-defence (anti-oxidant proteins)
Beta 1-4, endoglucanase	Hydrolase
Pectat-Lyase	Pecto-cellulosic cell wall hydolase

The final study's step is the obtention of mass fingerprint for the candidate proteins by mass spectrometry, in order to match proteins in databases or de novo sequencing if necessary.

The identification of quarantine root-knot nematodes that is a preliminary aspect of studies, management and phytosanitary controls is now possible and reliable with a serological test. The results of proteomics studies on virulence/avirulence of quarantine root-knot nematodes allow identification of virulent/avirulent population and enhancing knowledge upon the virulence/avirulence process to improve resistance crop management.

Quarantine root-knot nematodes in Europe awarness, resistance, management and phytosanitary policy Workshop.
9-10 october 2003, Wageningen, The Netherlands

Institut National de la Recherche Agronomique