



UNIVERSITÀ
CATTOLICA
del Sacro Cuore



XXIII Convegno Nazionale Società Italiana di Patologia Vegetale - SIPaV

In occasione del 25° anniversario dalla fondazione

Piacenza, 4-6 ottobre 2017

Università Cattolica del Sacro Cuore
Via Emilia Parmense, 84

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Macrophomina phaseolina is a soil- and seed-borne generalist fungal pathogen with a global distribution and can infect more than 500 plant species. In infected tissues the fungus produces microsclerotia which enable it to survive in soil for 2-15 years, act as primary inoculum source and are needed for the correct identification of the pathogen. The disease occurrence and severity are directly related to the population of viable microsclerotia in soil. The 'Nucleic Acid Lateral Flow Immunoassay' using a generic 'Lateral Flow Device', combined with PCR employing labelled primers to detect a specific amplicon, enables to circumvent the use of electrophoresis, making the diagnostic procedure faster and easier. This study describes the development of the species-specific primers MP102F/MP102R for *M. phaseolina* based on the intergenic spacer (IGS) of the rDNA sequence analysis. The primer specificity was checked and confirmed using 20 isolates of the pathogen and other 16 non-target fungi. Microsclerotia of *M. phaseolina* (1, 10, 100 and 200) were manipulated under a stereomicroscope and their DNA was extracted from microsclerotia alone or mixed with different types of soil. The resulting DNA, used in the PCR-NALFIA assay, provided positive results for all the samples tested. A semi-quantitative grey-scale reference card was developed using intervals corresponding to microsclerotia soil number. For this purpose, the normalized pixel grey volumes obtained after a densitometric analysis of the test line intensity generated by the LFD strips were used. Patent application relating to the method here described is pending.

TOWARDS A SUSTAINABLE STRATEGY FOR XYLELLA FASTIDIOSA CONTROL. G. D'Attoma^{1,2}, M. Morelli², S. Cicco³, M. Saponari², P. Saldarelli². ¹Università degli Studi di Bari Aldo Moro, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, 70126 Bari, Italy. ²CNR-Istituto per la Protezione Sostenibile delle Piante (IPSP), 70126 Bari, Italy. ³CNR-Istituto di Chimica dei Composti Organo Metallici (ICCOM), 70126 Bari, Italy. E-mail: giusy.dattoma@ipsp.cnr.it

Xylella fastidiosa (*Xf*) is a xylem-limited bacterium, regulated as a quarantine pest, that is causing a devastating disease on olive crops in the southern area of Apulia (Italy) and whose potential spread in the Mediterranean area poses a severe threat to EU agriculture and landscape environment. *Xf* virulence is related to the expression of a cluster of *rpf* (regulation of pathogenicity factors) genes responsible for a signalling system based on small fatty acid molecules called DSF (Diffusible Signalling Factor). Since DSF regulation is involved in pathogenicity traits of *Xf* and biofilm formation, a "pathogen-confusion" strategy, based on the alteration of DSF levels *in planta*, has been proposed to contrast bacterial infection. In grapevine, the strategy is based on the transgenic expression of the *rpfF* gene, which encodes the DSF-synthase. We are attempting to express the *rpfF* gene of the olive-infecting *Xf* strain CoDiRO in the heterologous *Escherichia coli* system. The gene product has been successfully detected by Western blot analysis in cell protein extracts. Chemical characterisation by Gas Chromatography-Mass Spectrometry analysis of the DSF molecules produced by this expression system, in addition to those naturally produced by *Xf* CoDiRO, are underway. Concurrently, a TMV-based vector has been engineered to harbour the same *rpfF* gene and induce its transient expression *in planta*. Biologically active transcripts of the vector have been inoculated to *Nicotiana tabacum* and *N. benthamiana* plants, to establish a model system on herbaceous hosts. *RpfF* expression was successfully proved by Western blot analysis, whereas movement and systemic colonisation of plant tissues were evaluated by RT-PCR assays. The same viral vector harbouring GFP in replacement of *rpfF* is used as a control. Following inoculation with *Xf* CoDiRO bacterial cells the system is now being tested to monitor the persistence of DSF expression and its efficacy to lower

disease susceptibility or movement of bacterial cells behind the point of inoculation.

A COMBINED APPLICATION OF A BIOPOLYMER AND TRICHODERMA – A PROMISING TOOL TO CONTROL ROOT-KNOT NEMATODES. G. d'Errico¹, F. Lacatena², N. Lombardi¹, G. Murolo³, C. Gigliotti³, G. Manganiello⁴, M. Malinconico⁴, P. Mormile⁵, S. Bolletti Censi³, A. Vassetti¹, F. Vinale^{1,2}. ¹Consiglio Nazionale delle Ricerche - Istituto per la Protezione Sostenibile delle Piante (CNR-IPSP), Via Università 133, 80055 Portici (NA). ²Università degli Studi di Napoli Federico II - Dipartimento di Agraria, Via Università 100, 80055 Portici (NA). ³Marea Scarl, Via Vittoria Colonna 14, 80121 Napoli. ⁴Consiglio Nazionale delle Ricerche - Istituto dei Polimeri, Compositi e Biomateriali (IPCB), Via Campi Flegrei 34, 80078 Pozzuoli (NA). ⁵Consiglio Nazionale delle Ricerche - Istituto di Scienze Applicate e Sistemi Intelligenti (ISASI), Via Campi Flegrei 34, 80078 Pozzuoli (NA). E-mail: francesco.vinale@ipsp.cnr.it

In recent years, there has been growing interest in the use of biopolymers for a wide range of agricultural applications. A polysaccharide-based compound may be a good carrier of biological factors or act as a barrier against pathogens due to its ability to form films coating plant structures. In the present study we evaluate the combination of a biopolymer and *Trichoderma* strains for the management of disease caused by the root-knot nematode *Meloidogyne incognita* on tomato. Previous experiments have indicated that *Trichoderma* species are able to act both as crop protection agent and plant growth promoters. A greenhouse test was performed by dipping the roots in an aqueous solution of the biopolymer, which were then air-dried prior to planting in either naturally infested or sterilized soil. Spore suspensions of selected *Trichoderma* strains were watered at the time of transplant and every two weeks for the next two months. Preliminary results indicate that root treatment with the polysaccharide mixture combined with the *Trichoderma* soil amendment, significantly improves plant growth and development in comparison to controls. Evaluation is ongoing to determine if the effect is due to inhibition of *M. incognita*, demonstrated by a decreased nematode infestation and reduction in galling, and/or increased plant general fitness. The applied biopolymer may function as a physical barrier against the nematode and/or as a substrate supporting the colonization of the rhizosphere by plant growth-promoting beneficial microbes.

Work supported by MAREA project (MIUR PON03PE_00106_1).

PEPTAIBOLS: NATURALLY OCCURRING PEPTIDES AS BIOFUNGICIDES. M. De Zotti¹, L. Sella², A. Bortolotto^{1,2}, I. Elmaghraby², F. Favaron¹. ¹Department of Chemistry, University of Padova, Via Marzolo 1, 35131 Padova, Italy. ²Department of Land, Environment, Agriculture and Forestry, University of Padova, Viale dell'Università 16, 35020, Legnaro (PD), Italy. E-mail: marta.dezotti@unipd.it

Fungi belonging to the genus *Trichoderma* are distributed worldwide and have been successfully used in field trials against many crop pathogens. They produce peptaibols, a peculiar family of peptides, as part of their defense system against other microorganisms. Such secondary metabolites are known for their plant-protection properties: they (i) possess antimicrobial activity, (ii) act as stimulants of plant defences and growth, (iii) elicit plant production of volatiles to attract natural enemies of herbivorous insects. Moreover, peptides are ecofriendly compounds that are degraded by enzymes to nontoxic amino acids. The large molecular diversity of