ISOLATION OF DIFFERENTIALLY EXPRESSED TRANSCRIPTS AFTER TREATMENT OF *PLATANUS ACERIFOLIA* LEAVES WITH CERATO-PLATANIN, A MULTI-FUNCTIONAL PROTEIN FROM *CERATOCYSTIS PLATANI*

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Cerato-platanin (CP) is a small protein of 120 amino acids, produced by the Ascomycete *Ceratocystis platani* (*Cep*), the causal agent of plane canker stain. *Cep* is pathogenic to *Platanus orientalis*, *P. occidentalis* and their hybrid *P. acerifolia*. This disease has had a dramatic impact on plane trees native to many European and Mediterranean countries where resistant host clones are not reported. CP has a molecular weight of about 12.4 kDa and contains four cysteine residues at positions 20, 57, 60 and 115, forming two S-S bridges (Pazzagli *et al., J. Biol. Chem.* **274**: 24959-24964, 1999). CP is located in the cell walls of the conidia, hyphae and ascospores of *Cep*, is able to self-aggregate, and is released into the medium since the very beginning of the *in vitro* fungal culture. When applied to host and non host leaves, CP elicits the synthesis of phenolic compounds and/or phytoalexins, causes intercellular and intracellular disorganization of spongy parenchyma cells, cell plasmolisis and/or necrosis (Scala *et al., J. Plant Pathol.* **86**: 23-29, 2004). Since the 1-119 amino acid sequence of CP has been defined a novel domain, called "cerato-platanin domain", this protein became the founder member of the cerato-platanin family. Suppression Subtractive Hybridization (SSH) is a powerful technique that isolates ESTs representing genes that are differentially expressed in different mRNA populations. The advantages of this technique include its ability to detect scarcely abundant, differentially expressed transcripts and to isolate genes without prior knowledge of their sequence or identity; moreover, SSH uses common molecular biological techniques that do not require specialized equipment or analysis. The aim of the present study was to characterize the expression profile of the genes modulated and/or activated by CP treatment so as to improve our understanding of the *Cep-P. acerifolia* interaction, and in particular, of the potential of CP to function as a PAMP (Pathogen-Associated Molecular Pattern) in the pathogenic process. We analysed sequences from the forward library sequencing 78 differential clones selected from among 576 clones screened. The putative differentially expressed genes were identified using the FASTA, BLASTN and BLASTX programs. The up-regulated clones were classified in 6 macro putative groups taking in account the functional categories established for *Arabidopsis* (The *Arabidopsis* Genome Initiative, *Nature* **408**: 796-815, 2000). We also analysed some clones by relative Reverse Transcription-PCR (RT-PCR) using total RNA extracted from *Cep* conidia or CP-treated leaves for 6, 24 and 48 h in order to investigate possible differences in gene expression between these systems (*Cep*-plant and CP-plant). Results showed that CP acted at low concentrations like some very active substances that are able to elicit defence responses in plants, such as, for example, the α-elicitins and the oligogalacturonides. From the relative RT-PCR results it emerged that CP succeeded in mimicking only some of the *Cep* actions, probably because it is not the only active fungal protein.

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A MOLECULAR METHOD TO ASSESS *PHYTOPHTHORA* DIVERSITY IN NATURAL AND SEMI-NATURAL ECOSYSTEMS

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A new molecular method to detect and identify members of the genus *Phytophthora* was developed and applied to investigate natural *Phytophthora* communities in soil and water samples. The method is based on a nested-PCR assay with new sets of genus-specific primers, designed on the internal transcribed spacer region (ITS1) of the ribosomal DNA gene (rDNA). PCR fragments, amplified from environmental DNA samples, were cloned in *Escherichia coli* and sequenced from a representative number of colonies. ITS1 sequences were then subjected to phylogenetic and diversity analysis in order to identify and discriminate the different *Phytophthora* species within the original sample. A major advance with respect to previously published methods was the lack of cross reaction with the ubiquitous *Pythium* species. The method was validated in the course of several surveys, during 2005 and 2006, collecting soil and water samples from a range of Scottish natural and planted woodlands. In particular, an in field filtration method was developed, for a more efficient capture of *Phytophthora* zoospores from naturally infested water samples. DNA extraction and purification procedures were optimised to obtain genomic DNA suitable for PCR amplification. Results showed a very efficient DNA amplification, particularly from water, and a great diversity of *Phytophthora* species in a range of different forest ecosystems, especially when compared with the conventional baiting tests. The resolution from only 260bp (ITS1 region) proved to be adequate to distinguish different *Phytophthora* species in heterogeneous environmental DNA samples and even for discriminating minor sequence ‘variants’ in a complex grouping of taxa, such as the ITS clade 6, which was the most frequently detected. The analysis of genetic distance revealed the presence of 23 known *Phytophthora* phylotypes, from all main ITS-clades of the genus (Cooke *et al. Fungal Genet. Biol.* **30**: 17-32, 2000): *P. cactorum, P. hedraiandra, P. nicotianae* (clade 1); *P. capsici* (clade 2); *P. quercina, P. pseudosyringae* (clade 3); *P. gonapodyides, P. megasperma, P. inundata, P.* taxon Oaksoil, *P.* taxon Salixsoil, *P.* taxon Pgchlamydo, *P.* taxon Raspberry (clade 6); *P. europaea, P. alni* subsp. *multiformis, P. cambivora, P. cinnamomi, P. niederhausierii, P. pistaciae* (clade 7); *P. syringae, P. cryptogea, P. drechsleri, P. hibernalis* (clade 8). Furthermore, three novel groups of ITS1 sequences, with no current match in GenBank, were also identified: *P. europaea*-like*, P. medicaginis*-like and *P. hedraiandra*-like. Their phylogenetic analysis strongly suggests the presence of yet undescribed species. The range and type of the sequences present varied from sample to sample and up to five different *Phytophthora* phylotypes were detected in a single sample.

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