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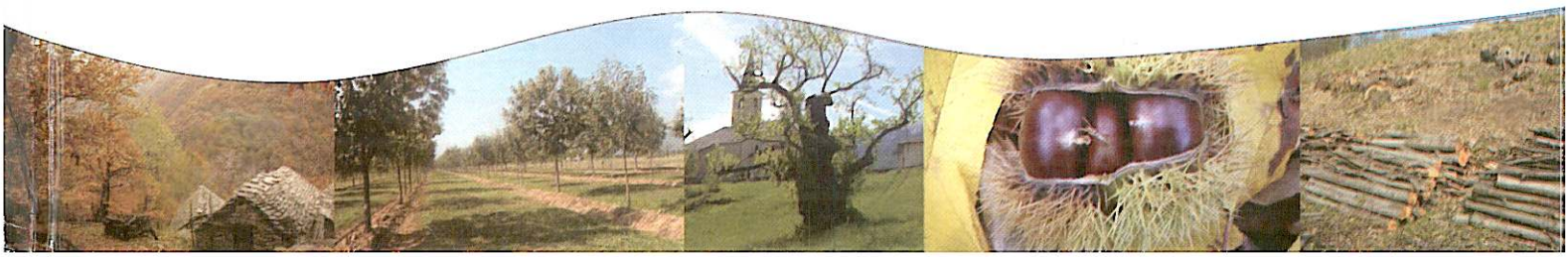
5° Convegno Nazionale Castagno

ABSTRACTS



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IDENTIFICATION AND CHARACTERIZATION OF MOLECULAR FACTORS ASSOCIATED WITH THE *Phytophthora cinnamomi* INFECTION MECHANISMS

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INTRODUCTION

Phytophthora species are the causal agents of many serious plant diseases. They secrete large amounts of elicitins, a group of unique highly conserved proteins that are able to induce hypersensitive response (HR) and enhance plant defense responses in a systemic acquired resistance (SAR) manner against infection by different pathogens. *Phytophthora cinnamomi,* one of the most destructive species of *Phytophthora* genus is the causal agent of *Castanea sativa* ink disease, and has been associated with the decline of several forest, ornamental and fruit trees and shrubs, causing enormous economic losses worldwide.

We briefly describe some of the proteins involved in *P. cinnamomi* infection mechanisms: a transglutaminase (TGase, induction of defense responses and disease-like symptoms), a glucanase inhibitor protein (GIP, causing suppression of host defense responses) and a necrosis-inducing protein 1 (NPP1).

MATERIALS AND METHODS

All genes referred in this study were obtained from *P. cinnamomi* strain Pr120 genomic DNA. Elucidation of complete gene nucleotide sequences of TGase, GIP and NPP1 were achieved by High-Efficiency Thermal Asymetric Interlaced PCR (HE-TAIL PCR), a method described by Michiels *et al.* (2003). DNA sequencing was performed using an ABI 373 automated sequencer. The open reading frames (ORF) of *P. cinnamomi genes* were identified by BioEdit program and submitted to EMBL databases (GIP-accession number CAJ90742.1; *P. cinnamomi* transglu gene for transglutaminase elicitor precursor, accession AM403129; *P. cinnamomi* npp1 gene for necrosis-inducing protein - accession AM403130). Nucleotide and amino acid sequences were analyzed using FASTA programs from EMBL databases. ClustalW2 (Larkin *et al*., 2007) was used to align the *Phytophthora* genus sequences.

RESULTS

The translated ORF of *P. cinnamomi* GIP codifies a 269aa protein, with a predict Mw of 28,8KDa. Scanning against protein search databases revealed that *P. cinnamomi* GIP are a serine protease, with a trypsin domain profile. A characteristic feature of Ser proteases is to have a catalytic triad charge relay system, with residues of H, D and S in that order along the sequence, essential for the proteolytic function.In Figure 1 are shown the multiple alignment of various sequences who showed great homology with *P. cinnamomi* GIP, including another GIPs of *Phytophthora* genus, and a serine protease and a trypsin protease from *P. infestans.* GIPs have in common the fact that none of them have an intact catalytic triad, like other serine proteases, although they share with them several stretches of amino acids and motifs that are highly conserved. Thus, in all *Phytophthora* GIPs, there are substitutions in residues of the catalytic triad: H-79→A,S,T,I,M-79 (in *P. cinnamomi:* S-79), D-128→N-128 (only in *P. cinnamomi* and *P. sojae* GIP2, and Ser-217→T-217, in all *Phytophthora* GIPs.Therefore, GIPs are proteolytically inactive, referred as serine protease homologs, and presumably function as host-enzyme inhibitors. It can be hypothesized that a major role for GIP is to suppress the release of glucan elicitors during *Phytophthora sp.* infections, thereby reducing the effectiveness of the plant host's surveillance system (Rose *et al*., 2002).

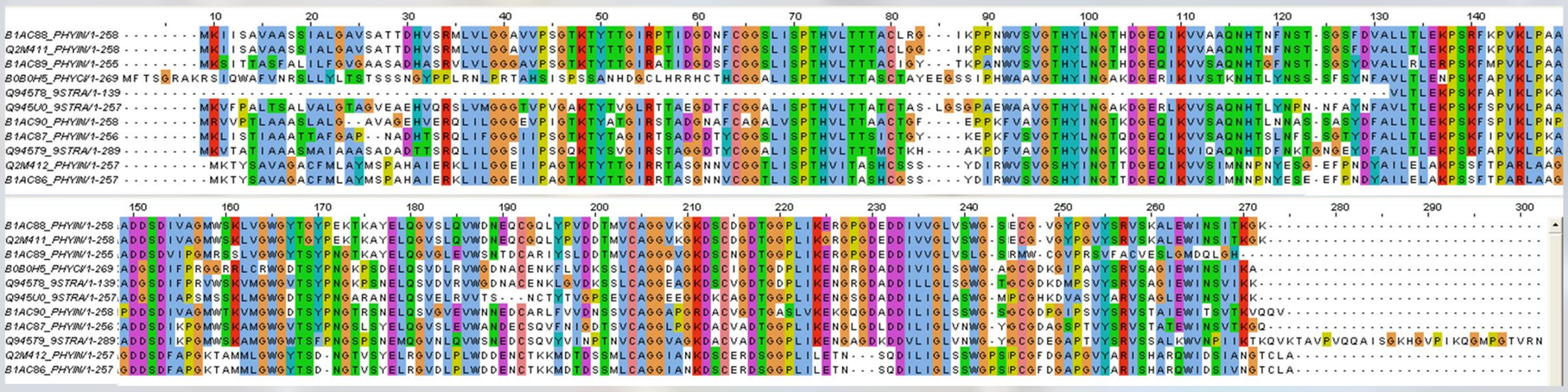

FIGURE 1 – Multiple sequence alignment of GIP and GIP-like genes from *Phytophthora* sp.

B0B0H5_PHYCIN – putative GIP from *P. cinnamomi*; Q945U0_9STRA – GIP1 from *P.sojae*; B1AC90_PHYIN – GIP1 from *P.infestans*; Q945T9_9STRA – GIP2 from *P.sojae*; B1AC87_PHYIN – GIP2 from *P.infestans*; Q945T8_9STRA - GIP2 from *P.sojae*; B1AC88_PHYIN – GIP3 from *P.infestans*; Q2M411_PHYIN – trypsin protease GIP-like; B1AC89_ PHYIN – GIP4 from *P.infestans;* B1AC86_ PHYIN – serine protease from *P.infestans*; Q2M412_PHYIN- trypsin protease GIP-like.

P. cinnamomi transglutaminase is a protein with 533 aa with a predict Mw of 57,7KDa. P*hytophthora* sp.TGases are even more closely related amongst them than GIPs, as shown in Figure 2.

P. cinnamomi necrosis-inducing protein1 has 256 aa with a predict Mw of 29,0KDa. Scanning against protein search databases (data not shown) revealed that sequences who showed greater homology with *P. cinnamomi* NPP1 were two NPP1 proteins of *P. infestans (Q2M430 _PHYIN; Q2M429 _PHYIN).* The phylogram showing the more closely related sequences are shown in Figure 3.

FIGURE 2 – Multiple sequence alignment of TGase genes from *Phytophthora* sp. B0B0Q5_PHYCI - *P. cinnamomi* transglutaminase elicitor; Q6Q475_9STRA – *P. sojae* transglutaminase elicitor; Q01928_PHYME – *P. megasperma* glycoprotein elicitor; Q6XDM3_PHYIN - *P. infestans* transglutaminase elicitor M81C; Q6XDM2_PHYIN - *P. infestans* transglutaminase elicitor M81D)

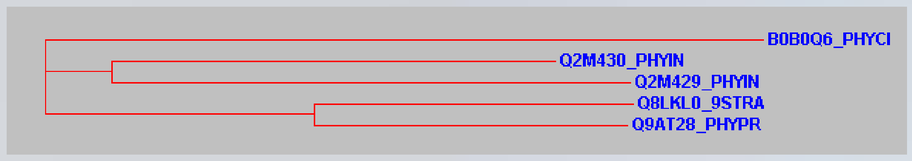

FIGURE 3 – Phylogram of most closely related *P. cinnamomi* NPP1 protein. B0B0Q6_PHYCI - *P. cinnamomi* NPP1; Q8LKL0_9STRA – *P. sojae* necrosis-inducing-like protein; Q9AT28_PHYPR – *P. parasitica* necrosis-inducing-like protein; Q2M430_PHYIN - *P. infestans* NPP-like protein; Q2M429_PHYIN - *P. infestans* NPP-like protein.

Further studies, including plant-pathogen phenotypic interactions are needed to understand how each individual factor can affect pathogenesis mechanisms of P. *cinnamomi,* and how this knowledge can be used for control ink disease and other *Phytophthora* sp. diseases .

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