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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 elicitors, 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 Casutanea 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 processes 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 asymmetric 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 CAJ07421; P. cinnamomi transglue 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 GTPs 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 protease sequences, although they share with them several stretches of amino acids and motifs that are highly conserved. Thus, in all Phytophthora GTPs, there are substitutions in residues of the catalytic triad: H79→A, S179→T9 (in P. cinnamomi: S79), D128→N→D128 (only in P. cinnamomi and P. sojae GIP2), and Ser217→T121, in all Phytophthora GTPs. Therefore, GPs 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.

P. cinnamomi transglutaminase is a protein with 533 aa with a predict Mw of 57.7KDa. Phytophthora 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.

FIGURE 3 – Phylogram of most closely related P. cinnamomi NPP1 protein.

P. cinnamomi necrosis-inducing protein1 protein will be 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.

REFERENCES


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