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Centro de Investigação Montanha 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 \rightarrow A,S,T,I,M-79 (in *P. cinnamomi*: S-79), D-128 \rightarrow N-128 (only in *P. cinnamomi* and *P. sojae* GIP2, and Ser-217 \rightarrow 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).

Q2M411_PHYIW/1-258	AS <mark>SIALGAVSATTDHVSR</mark> ML FALILFGVGAASADHASRVL NR <mark>SLLYLTS</mark> TSSSNGYPPLR SALVALGTAGVEAEHVQRSL AASLALG-AVAGEHVERQL AATTAFGAP-NADHTSRQL AASMAIAAASADADTTSRQL	VLGGAVVPSGTKTYTTGIRP VLGGGAVPSGTKTYTTGIRA NLPRTAHSISPSSANHDGCLI VMGGGTVPVGAKTYTVGLRT ILGGGEVPIGTKTYATGIRS IFGGGIIPSGTKTYTAGIRT ILGGEIIPSGQKTYSVGIRS	TIDGDNFCGGSLISPTHVLT TIDGDSFCGGSLISPTHVLT HRRHCTHCGGALISPTHVLT TAEGDTFCGGALISPTHVLT TADGNAFCAGALVSPTHVLT SADGDTYCGGSLISPTHVLT	TTACLGG IKPPNWVSVG TTACLGY TKPANWVSVG TTASCTAYEEGSSIPHWAAVG TTATCTAS - LGSGPAEWAAVG TTAACTGF EPPKFVAVG TTSICTGY KEPKFVSVG	THYLNGTHDGEQIKVVAAQNI THYLNGTHDGEQIKVVSAQNI THYINGAKDGERIKIVSTKNI THYLNGTKDGEQIKVVSAQNI THYLNGTKDGEQIKVVSAQNI THYLNGTQDGEQIKVVSAQNI	120 130 140 140 150 170 170 170 170 170 170 170 17
150 160 B1AC88_PHYIW1-258 ADD SD I VAG MWS KLVGWO Q2M411_PHYIW1-258 ADD SD I VAG MWS KLVGWO B1AC89_PHYIW1-255 ADD SD V I PGMRS SLVGWO B0B0H5_PHYCW1-269 ADG SD I F P R GG R R L C RWO Q94578_9STRA/1-139 ADG SD I F P R VWS KVMGWO Q94500_9STRA/1-257 ADG SD I F P R VWS KVMGWO B1AC90_PHYIW1-258 P DD SD I VAG MWT KVMGWO B1AC87_PHYIW1-256 ADD SD I VAG MWS KAMGWO	170 GYTGYPEKTKAYELQGVSLQV GYTGYPEKTKAYELQGVSLQV GYTSYPNGTKAYELQGVGLEV GDTSYPNGKPSDELQSVDLRV GVTSYPNGKPSNELQSVDLRV GDTSYPNGTRSNELQSVELRV GDTSYPNGTRSNELQSVGVEV GVTSYPNGSLSYELQGVSLEV GWTSFPNGSPSNEMQGVNLQV GYTSD-NGTVSYELRGVDLPL	190 200 WD NEQCGQL YPVD D TMVCAGG WD NEQCGQL YPVD D TMVCAGG WS NTD CARIYSLD D TMVCAGG WG DNACENKFLVD KSSLCAGG WG DNACENKLGVD KSSLCAGG WG DNACENKLGVD KSSLCAGG WG DNACENKLGVD KSSLCAGG WG DNACENKLGVD KSSLCAGG WS NEDCARLFVVD NSSVCAGG WAND ECSQVFNIGD TSVCAGG WS NEDCSQVYVINPTNVCAGG WD DENCTKKMD TD SSMLCAGG	VKGKDSCDGDTGGPLIKGRGF GVGKDSCNGDTGGPLIKERGF DAGKDSCVGDTGDPLIKENGF EAGKDSCVGDTGDPLIKENGF APGRDACVGDTGGPLIKENGF UPGKDACVADTGGPLIKENGF VAGKDACVADTGGPLIKENGF	PGDEDDIVVGLVSWG-SECG PGDEDDIVVGLVSLG-SIRMW RGDADDIVIGLSGWG-AGCCD RGDADDILLGLSGWG-TGCGD SGDADDILLGLSSWG-MPCGH QGDADDILIGLSSWG-SGCGD LGDLDDILIGLVNWG-YGCGD SQDILIGLSWGPSPCGF	VGYPGVYSRVSKALEWINSITK CGVPRSVFACVESLGMDQLGH KGIPAVYSRVSAGIEWINSIIK KDMPSVYSRVSAGIEWINSVIK KDVASVYARVSAGLEWINSVIK PGIPSVYSRVSTATEWITSVTK AGSPTVYSRVSTATEWINSVTK EGAPTVYSRVSSALKWVNPIIK DGAPGVYARISHARQWIDSIAN	G A K VQQV GQ TKQVKTAVPVQQAISGKHGVPIKQGMPGTVRN GTCLA

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. Phytophthora sp.TGases are even more closely related amongst them than GIPs, as shown in Figure 2.

P. cinnamomi necrosis-inducing protein1 has 256 as 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.

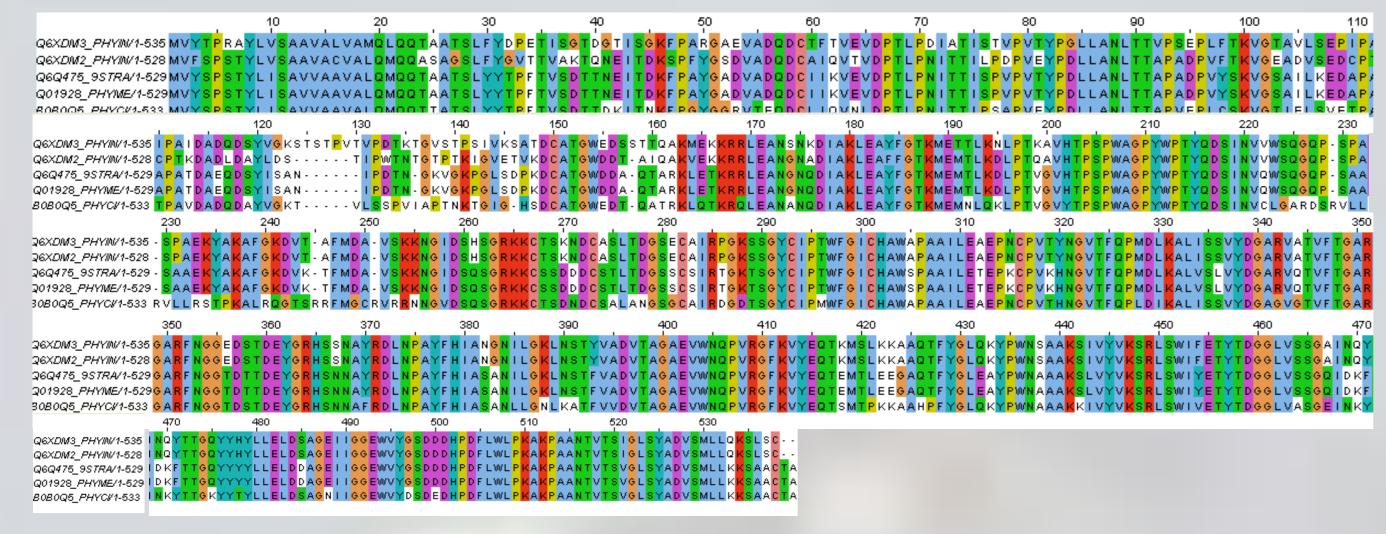


FIGURE 2 – Multiple sequence alignment of TGase genes from

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.

REFERENCES

Larkin, M., Blackshields, G., Brown, N., Chenna, R., McGettigan, P., McWilliam, H., Valentin, F., Wallace, I., Wilm, A., Lopez, R., Thompson, J., Gibson, T. & Higgins D., 2007. Bioinformatics, 23(21): 2947-2948. Michiels, A., Tucker, M., Van Den Ende, W. & Van Laere, A., 2003. Chromosomal Walking of Flanking Regions From Short Known Sequences in GC-Rich Plant Genomic DNA. *Plant Molecular Biology Reporter*, 21:295-302. Rose, J., Ham, K., Darvill, A. & Albersheim, P., 2002. Molecular cloning and characterization of glucanase inhibitor proteins: coevolution of a counterdefense mechanism by plant pathogens. *Plant Cell*, 14:1329-1345.

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