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IDENTIFICATION OF A Phytophthora cinnamomi GLUCANASE INHIBITOR PROTEIN: A MOLECULAR FACTOR ASSOCIATED TO INFECTION MECHANISM



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INTRODUCTION

P. cinnamomi is the causal agent of Castanea sativa ink disease, one of the most destructive species of Phytophthora genus, and has been associated with the decline of several forest, ornamental and fruit trees and shrubs, causing enormous economic losses worldwide.

Plant endo-\beta-1,3-glucanases and chitinases inhibit the growth of some fungi and generate elicitor-active oligosaccharides while depolymerizing polysaccharides of mycelial walls. Phytophthora cell walls are composed of glucans and have no chitin. Production of glucanase inhibitor proteins (GIPs) by Phytophthora species are thought to provide them a counterdefense against plant hosts β-1,3-glucanases (Rose et al., 2002), that otherwise would degrade these pathogen cell walls.

We report the identification of a gene encoding the first known *P. cinnamomi* GIP, presumably involved in the pathogen infection mechanism.

MATERIALS AND METHODS

Genomic DNA was obtained from strain *P. cinnamomi* Pr120 as described by Cooke and Duncan

TABLE 1 - HE-TAIL PCR cycle settings and conditions*

(1997). Polymerase chain reaction was used to amplify a 308bp fragment of the GIP gene, using degenerate oligonucleotide primers - M1 (5'-GAGAAGCCCAGCAAGTTCSCC-3') and M3 (5'-GCSGACACGCGMGMGTACACA-3'), which were designed based on homology of previous published *Phytophthora sp.* GIP's sequences from EMBL databases. PCR was performed with 10X amplification buffer; 100ng DNA template; 0.2 mM dATP, dCTP, dGTP, and dTTP (each); 0.2µM each primer; 1.5mM MgCl2; and 1U Taq DNA polymerase (Promega), in a final reaction volume of 50 µl. Amplification consisted of: one cycle of 5 min at 94°C, and 36 cycles of denaturing for 1 min at 94°C; annealing for 1 min at 63°C; and extension for 30 s at 72°C. A final extension step of 5 min at 72°C was done for one cycle. Full gene sequence length elucidation (1171bp) was achieved by flanking the previous known sequence with asymmetric HE-TAIL PCR using the degenerated primers R1, R2, R3 and R4 and the methodology described by Michiels et al. (2003), and the gene-(5'-GCCGTTYTCCTTGATCAGCGG-3'), M3, M2 specific M1, (5'primers **S1** AGGCGTTGTCGCCCCAGACC-3'), S2 (5'-CGGCCGCGGTGACGCTGACG-3') and S4 (5'-GGTCTGGGGGGGACAACGCCT-3') (Figure 1),. Three rounds of PCR were performed on a MyCycler Thermal Cycler (BIORAD), using the product of the previous PCR as a template for the next. A detailed cycler program and conditions are given in Table1.

DNA sequencing was performed using an ABI 373 automated sequencer. The open reading frame

Reaction	Number of Cycles	Thermal Settings
Prim ary		
	1	93°C (1min); 95°C (5min)
	5	94°C (30seg), 62°C (1min), 72°C (2min30s)
	1	94°C (30seg), 25°c ramping 72°C (3min); 72°C (2min30s)
	15	94°C (20s), 65°C (3min30s);
		94°C (20s), 65°C (3min30s);
		94°C (30s), 42°C (1min), 72°C (2min30s)
	1	72°C (5min), 4°C Hold
Secondary		
	12	94°C (20s); 65°C (3min30s);
		94°C (20s); 65°C (3min30s);
		94°C (30s); 42°C (1min); 72°C (2min30s)
	1	72°C (5min), 4°C Hold
Tertiary		
	30	94°C (30s); 42°C (1min); 72°C (2min30s)
	1	72°C (5min); 4°C Hold

*The primary PCR was performed in a 50μl volume containing 80ng of genomic DNA; 0.2 μM of primers M1 or M3; 2 μM of a random primer (R1,R2,R3,R4); 0,2mM of each dNTP; 1U Taq DNA polymerase (Promega) and 10X amplification buffer supplied with the enzyme. The secondary PCR was performed with primers M2 or S4 (0.2 μ M) and the same random primer R (2 μ M) as used in the primary reaction. One microliter of 1/50 dilution of the primary PCR was used as a template. Single-step annealingextension PCR consisting of a combined annealing and extension step at 62°C or 65°C was used in primary and secondary PCR reactions. The tertiary reaction was carried out with 1 µl of 1/10 dilution of the secondary reaction, 0.2 µM of primers S1 and S2, 0.2 μM of random primer R (the same as used in the previous cycles), 0.2 mM of each dNTP, 1U DNA Taq polymerase (Promega) and 10X amplification buffer. To exclude nonspecific amplification, a tertiary control reaction R-R was set up without adding gene-specific primers.



(ORF) of *P. cinnamomi* GIP was identified by BioEdit program and submitted to EMBL databases (Accession number CAJ90742.1); 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 GIP sequences.

RESULTS AND DISCUSSION

The translated ORF of *P. cinnamomi* GIP codifies a 269aa protein, with a predict Mw of 28,8KDa and a theoretical iso-electric point of 8,54. 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 2 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.

FIGURE 1 - Schematic representation of TAIL-PCR amplification.

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).

	10	20	30	40	50	60	70	80	90	100	110	120	130	140	
B1AC88 PHYIN/1-25	8 MKIISA	VAASSIALGAV	SATTDHVSRM	LVLGGAVVPS	GTKTYTTGIRP	TIDGDNFCGGS	ISPTHVLTTT		PNWVSVGTHY	LNGTHDGEQ	KVVAAQNHT	NFNST-	SGSFDVALLT	LEKPSRFKP	VKLPAA
Q2M411 PHYIN/1-28	8 M <mark>K</mark> IISA	VAASSIALGAV	SATTDHVSRM	ILVLGGAVVPS	GTKTYTTGIRP	TIDGDNFCGGS	ISPTHVLTTT	CLGG · · · IKP	PNWVSVGTHY	LNGTHDGEQ	IKVVAAQNHT	NFNST-	SGSFDVALLT	LEKPSRFKP	VKLPAA
BIAC89 PHYIN/1-25	5 M <mark>K</mark> SIT <mark>T</mark>	ASFALILFOVG	AASADHASRV	LVLGGGAVPS	GTKTYTTGIRA	TIDGDSFCGGS	. I SPTHVLTTT	CIGY TKP	ANWVSVGTHY	LNGTHDGEQ	IKVVSAQNHT	GFNST-	SGSYDVALLR	LERPSKEMP	VKLPAA
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Q94578 9STRA/1-13	39												VL T	LEKPSKFAP	IKLPKA
Q945U0 9STRA/1-2	57 MKVFPA	LTSALVALGTA	GVEAEHVQRS	LVMGGGTVPV	GAKTYTVGLRT	TAEGDTFCGGA	ISPTHVLTTA	CTAS - LGSGP	AEWAAVGTHY	LNGAKDGER	LKVVSAQNHT		NFAYNFAVLT	LEKPSKFSP	VKLPAA
BIAC90 PHYIN/1-25	8 M <mark>R</mark> VVPT	LAAASLALG	AVAGEHVERQ	LILGGGEVPI	GTKTYATGIRS	TADGNAFCAGA	VSPTHVLTTAA	CTGF···EP	PKEVAVGTHY	INGTKDGEQ	IKVVSAQNHT	LNNAS-	SASYDFALLT	LEKPSKFSP	IKLPNP
B1AC87 PHYIN/1-25	і6 M <mark>K</mark> L IS <mark>T</mark>	IAAATTAFGAP	· · NADHTSRQ	LIFGGGIIPS	GTKTYTAGIRT	SADGDTYCGGS	ISPTHVLTTS	С Т G Y • • • • К Е	PKFVSVGTHY	LNGTQDGEQ	IKVVSADNHT	SLNFS-	SGTYDFALLT	LEKPSKFIP	VKLPKA
Q94579 9STRA/1-28	89 · · · · · · · · MKVTAT	IAAASMAIAAA	SADADTTSRQ	LILGGSIIPS	GQ <mark>KTYS</mark> VG I RS	TAGGDTYCGGA	ISPTHVLTTTN		PDEVAVGTHY	VNGTKDGEQ	LKVIQAQNHI	DFNKTG	NGEYDFALLT	LEKPSKFAP	VKLPKA
Q2M412 PHYIN/1-28	7 MKTY <mark>s</mark>	AVAGACEMLAY	MSPAHAIERK	LILGGEIIPA	GTKTYTTGIRR	TASGNNVCGGT	ISPTHVITAS	ICSSSYD	IRWVSVGSHY	INGTTDGEQ	IKVVSIMNNF	NYESG -	EFPNDYAILE	LAKPSSFTP	ARLAAG
B1AC86_PHYIN/1-25	7 MKTY <mark>s</mark>	AVAGACEMLAY	MSPAHAIE <mark>R</mark> K	LILGGEIIPA	GTKTYTTG I R R	TASGNNVCGGT	. I SPTHVITASH	CGSSYD	IRWVSVGSHY	INGTTDGEQ	IKVVSIMNNF	NYESE-	EFPNDYAILE	LAKPSSFTP	ARLAAG
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B1AC88_PHYIW1-258 Q2M411_PHYIW1-258 B1AC89 PHYIW1-258	150 160 BIADDSDIVAGMWSKLV BIADDSDIVAGMWSKLV SIADDSDVIPGMRSSLV	170 / <mark>GWGYTGYP</mark> EK <mark>T</mark> /GWGYTGYPEKT /GWGYTSYPNGT	180 KAYELQGVSLC KAYELQGVSLC KAYELQGVGLE	190 IVWDNEQCGQL IVWDNEQCGQL VWSNTDCARI	200 YPVDDTMVCAGG YPVDDTMVCAGG YSLDDTMVCAGG	210 VKGKDSCDGDT VKGKDSCDGDT GVGKDSCNGDT	220 230 GPLIKERGPGDE GPLIKERGPGDE	D 240 D I VVG L VSW D D I VVG L VSW D D I VVG L VSL	250 3 - SIECG - VGY 3 - SIECG - VGY 3 - SIRMW - CGV	260 GVYSRVSKA GVYSRVSKA SVFACVES	270 EWINSITKG EWINSITKG GMDQLGH··	к' к	280	290	300 •
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B1AC88_PHYIW/1-258 Q2M411_PHYIW/1-258 B1AC89_PHYIW/1-258 B0B0H5_PHYCV1-268 Q94578_9STRA/1-138 Q945U0_9STRA/1-138 B1AC90_PHYIW/1-258 B1AC87_PHYIW/1-258	150 150 150 160 150 150 150 150 150 150 150 15	170 / GWGYTGYPEKT / GWGYTGYPEKT / GWGYTSYPNGT RWGDTSYPNGK / GWGDTSYPNGA / GWGDTSYPNGA / GWGDTSYPNGT / GWGVTSYPNGS	180 KAYELQGVSLQ KAYELQGVSLQ KAYELQGVGLE PSDELQSVDLF PSNELQSVDVF RANELQSVELF RSNELQSVGVE	190 VWDNEQCGQL VWDNEQCGQL VWSNTDCARI VWGDNACENK VWGDNACENK VVGDNACENK VVTSNCT VWNNEDCARL VWANDECSQV	200 YPVDDTMVCAGG YPVDDTMVCAGG YSLDDTMVCAGG FLVDKSSLCAGG LGVDKSSLCAGG YTVGPSEVCAGG FVVDNSSVCAGG	210 VKGKDSCDGDTG VKGKDSCDGDTG GVGKDSCNGDTG GAGKDSCIGDTG EAGKDSCVGDTG EEGKDKCAGDTG APGRDACVGDTG	220 230 GPLIKERGPGDE GPLIKERGPGDE DPLIKERGPGDE DPLIKENGRGDA GPLIKENGSGDA ASLVKEKGQGDA	D 240	250 3 • SIECG • VGY 3 • SIECG • VGY 3 • SIECG • VGY 3 • SIRMW • CGV 3 • AGCGDKGI 4 • GCGDKGI 5 • TGCGDKDM 5 • MPCGHKDV 3 • SGCGDPGI 5 • YGCGDAGS	260 GVYSRVSKA GVYSRVSKA RSVFACVES AVYSRVSAG SVYSRVSAG SVYSRVSAG SVYSRVSTA	270 EWINSITKG GMDQLGH EWINSITKA EWINSVIKK EWINSVIKK EWINSVIKK IEWINSVIKK	к к qqv	280	290	300
B1AC88_PHYIN/1-258 Q2M411_PHYIN/1-258 B1AC89_PHYIN/1-258 B0B0H5_PHYCI/1-268 Q94578_9STRA/1-258 B1AC90_PHYIN/1-258 B1AC87_PHYIN/1-258 Q94579_9STRA/1-288	150 150 150 160 150 150 150 150 150 150 150 15	170 / GWGYTGYPEKT / GWGYTGYPEKT / GWGYTSYPNGT RWGDTSYPNGK / GWGDTSYPNGA / GWGDTSYPNGT / GWGVTSYPNGS / GWGVTSFPNGS	180 KAYELQGVSLQ KAYELQGVGLE PSDELQSVDLF PSNELQSVDVF RANELQSVELF RSNELQSVGVE LSYELQGVSLE PSNEMQGVNLQ	190 VWDNEQCGQL VWDNEQCGQL VWSNTDCARI VWGDNACENK VWGDNACENK VVGDNACENK VVGDNACENK VVGDNACENK VVGDNACENK VVGDNACENK VVGDNACENK VVGDNACENK	200 YPVDDTMVCAGG YPVDDTMVCAGG YSLDDTMVCAGG FLVDKSSLCAGG LGVDKSSLCAGG YTVGPSEVCAGG FVVDNSSVCAGG FNIGDTSVCAGG	210 VKGKDSCDGDTG VKGKDSCDGDTG GVGKDSCNGDTG DAGKDSCVGDTG EAGKDSCVGDTG APGRDACVGDTG VAGKDACVADTG	220 230 G P L I KE R G P G D B G P L I KG R G P G D B G P L I KE R G P G D B D P L I KE N G R G D A D P L I KE N G R G D A G P L I KE N G S G D A G P L I KE N G L G D A G P L I KE N G L G D A	D 240	250 3 • SIECG • VGY 3 • SIECG • VGY 3 • SIECG • VGY 5 • SIECG • VGY 6 • VGY 6 • SIECG • VGY 6 • VGY 6 • VGY 6 • SIECG • VGY 6 • VGY 7 • VGY 6 • VGY 7 • VGY	260 GVYSRVSKA GVYSRVSKA RSVFACVES AVYSRVSAG SVYSRVSAG SVYSRVSAG SVYSRVSTA TVYSRVSTA	270 EWINSITKG GMDQLGH IEWINSIIKA IEWINSVIKK EWINSVIKK IEWINSVIKK IEWINSVIKK IEWINSVIKG	K K QQV QQV Q	280	290 , , , , , , , , , , , , , , , , , , ,	300
B1AC88_PHYIN/1-258 Q2M411_PHYIN/1-258 B1AC89_PHYIN/1-258 B0B0H5_PHYC/1-268 Q94578_95TRA/1-258 B1AC90_PHYIN/1-258 B1AC90_PHYIN/1-258 B1AC87_PHYIN/1-258 Q94579_95TRA/1-288 Q2M412_PHYIN/1-258	150 150 150 150 150 150 150 150	170 / GWGYTGYPEKT / GWGYTGYPEKT / GWGYTSYPNGT RWGDTSYPNGK A GWGVTSYPNGA A GWGDTSYPNGA A GWGVTSYPNGS A GWGVTSFPNGS GWGYTSD-NGT	180 KAYELQGVSLQ KAYELQGVSLQ KAYELQGVGLE PSDELQSVDLF PSNELQSVDVF RANELQSVGVE RSNELQSVGVE LSYELQGVSLE PSNEMQGVNLQ	190 VWDNEQCGQL VWDNEQCGQL VWSNTDCARI VWGDNACENK VWGDNACENK VWGDNACENK VWGDNACENK VWNNEDCARL VWNNEDCSQV VWSNEDCSQV	200 YPVDDTMVCAGG YPVDDTMVCAGG YSLDDTMVCAGG FLVDKSSLCAGG LGVDKSSLCAGG YTVGPSEVCAGG FVVDNSSVCAGG FVVDNSSVCAGG FNIGDTSVCAGG YVINPTNVCAGG	210 VKGKDSCDGDTG GVGKDSCNGDTG GVGKDSCNGDTG GVGKDSCNGDTG GVGKDSCVGDTG EEGKDKCAGDTG APGRDACVGDTG APGRDACVADTG VAGKDACVADTG	220 230 240 240 240 240 240 240 240 24	D I VVG L V SW D I VVG L V SW D I VVG L V SW D I VVG L V SL D I VVG L V SL SW D I L I G L S SW D I L I G L S SW D I L I G L V NW C D V L I G L V NW	250 3 • SIECG • VG YF 3 • SIECG • VG YF 3 • SIRMW • CG VF 3 • SIRMW • CG VF 3 • AG CG DKG IF 3 • TG CG DKDMF 3 • MPCG HKD VF 3 • SG CG DPG IF 3 • YG CG DAG SF 3 • YG CG DEG AF 3 • YG CG DEG AF	260 C VYSRVSKA C VYSRVSKA C VYSRVSKA C VYSRVSAG S VYSRVSAG S VYSRVSAG S VYSRVSAG S VYSRVSAG T VYSRVSAA T VYSRVSAA C VYAR I SHA	270 EWINSITKG GMDQLGH EWINSITKG EWINSVIKK EWINSVIKK EWINSVIKK EWINSVIKK EWINSVIKG EWINSVIKG	K K QQV QQV KQVKTAV	280 	290 , , , , , , , , , , , , , , , , , , ,	300
B1AC88_PHYIW/1-258 Q2M411_PHYIW/1-258 B1AC89_PHYIW/1-258 B0B0H5_PHYCV/1-268 Q94578_9STRA/1-138 Q945U0_9STRA/1-258 B1AC90_PHYIW/1-258 B1AC87_PHYIW/1-258 Q94579_9STRA/1-288 Q2M412_PHYIW/1-258 B1AC86_PHYIW/1-258	150 150 150 150 150 150 150 150	170 GWGYTGYPEKT GWGYTGYPEKT GWGYTSYPNGT RWGDTSYPNGK AGWGDTSYPNGK AGWGDTSYPNGA AGWGDTSYPNGA AGWGVTSYPNGS AGWGYTSPNGS GWGYTSD-NGT GWGYTSD-NGT	180 KAYELQGVSLQ KAYELQGVSLQ KAYELQGVGLE PSDELQSVDLF PSNELQSVDLF RSNELQSVGVE CSYELQGVSLE PSNELQGVSLE VSYELQGVDLF	190 VWDNEQCGQL VWDNEQCGQL VWSNTDCARI VWGDNACENK VWGDNACENK VWGDNACENK VWGDNACENK VWGDNACENK VWNNEDCARL VWANDECSQV LWDDENCTKK	200 YPVDDTMVCAGG YSLDDTMVCAGG FLVDKSSLCAGG GVDKSSLCAGG YTVGPSEVCAGG FVVDNSSVCAGG FNIGDTSVCAGG YVINPTNVCAGG MDTDSSMLCAGG	210 VKGKDSCDGDTG GVGKDSCDGDTG GVGKDSCNGDTG GAGKDSCVGDTG EEGKDKCAGDTG APGRDACVGDTG APGRDACVGDTG VAGKDACVADTG VAGKDACVADTG IANKDSCERDSG	220 230 G P L I KE R G P G D B G P L I KG R G P G D B G P L I KE R G P G D B D P L I KE N G R G D A G P L I KE N G R G D A G P L I KE N G L G D A G P L I KE N G L G D A G P L I L E T N S G P L I L E T N S	240 200 </td <td>250 3 - SIECG - VGYF 3 - SIECG - VGYF 3 - SIECG - VGYF 3 - SIRMW - CGVF 3 - AGCGDKGIF 3 - TGCGDKGIF 3 - TGCGDKDMF 3 - MPCGHKDV 3 - SGCGDPGIF 3 - YGCGDAGSF 3 - YGCGDEGAF 3 - YGCGFDAA</td> <td>260 G VYSR VSKA G VYSR VSKA R SVFACVES AVYSR VSAG SVYSR VSAG SVYSR VSAG SVYSR VSAG SVYSR VSAG TVYSR VSAA TVYSR VSAA G VYAR I SHA</td> <td>270 EWINSITKG EWINSITKG GMDQLGH EWINSVIKK EWINSVIKK EWINSVIKK EWINSVTKG EWINSVTKG EWINSVTKG RQWIDSINNG</td> <td>K K Q KQVKTAV TCLA</td> <td>280 , , , , , , , , , , , , , , , , , , ,</td> <td>290 , GVPIKQGMPG</td> <td>300 </td>	250 3 - SIECG - VGYF 3 - SIECG - VGYF 3 - SIECG - VGYF 3 - SIRMW - CGVF 3 - AGCGDKGIF 3 - TGCGDKGIF 3 - TGCGDKDMF 3 - MPCGHKDV 3 - SGCGDPGIF 3 - YGCGDAGSF 3 - YGCGDEGAF 3 - YGCGFDAA	260 G VYSR VSKA G VYSR VSKA R SVFACVES AVYSR VSAG SVYSR VSAG SVYSR VSAG SVYSR VSAG SVYSR VSAG TVYSR VSAA TVYSR VSAA G VYAR I SHA	270 EWINSITKG EWINSITKG GMDQLGH EWINSVIKK EWINSVIKK EWINSVIKK EWINSVTKG EWINSVTKG EWINSVTKG RQWIDSINNG	K K Q KQVKTAV TCLA	280 , , , , , , , , , , , , , , , , , , ,	290 , GVPIKQGMPG	300

FIGURE 2 – 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.

REFERENCES

Cooke, D. & Duncan, J., 1997. Phylogenetic analysis of *Phytophthora* species based on the ITS1 and ITS2 sequences of ribossomal DNA. Mycological Research, 101:667-677.

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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