Characterization of the dry bean polygalacturonase-inhibiting protein (PGIP) gene family during *Sclerotinia sclerotiorum* (Sclerotiniaceae) infection

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ABSTRACT. Polygalacturonase-inhibiting proteins are leucine-rich repeat proteins that inhibit fungal endopolygalacturonases. The interaction of polygalacturonase-inhibiting protein with endopolygalacturonases limits the destructive potential of endopolygalacturonases and may trigger plant defense responses induced by oligogalacturonides. We examined the expression of fungal *pg* and plant *Pvpgip* genes in bean (*Phaseolus vulgaris*) stems infected with *Sclerotinia sclerotiorum* to determine whether any of them are associated with the infection process. Transcriptional analysis was carried out by means of semi-quantitative reverse transcription PCR or real-time PCR. The *sspg1* gene was highly expressed during infection; *sspg3* was regulated during the later phases of infection; *sspg5* was more uniformly expressed during infection, whereas *sspg6* was only weakly expressed. During the course of infection, *Pvpgip1* transcripts were not detected at early stages, but they appeared 72 h post-inoculation. High levels of *Pvpgip2* expression were observed during the initial phase of infec-

tion; the transcript peaked by 48 h post-inoculation and declined by 72 h post-inoculation. *Pvpgip3* expression increased strongly at 96 h post-inoculation. *Pvpgip4* was constantly present from 24 h post-inoculation until the end of the experiment. However, we detected higher levels of the *Pvpgip4* transcript in the necrotic lesion area than in plants that had been mechanically wounded. Remarkably, only *Pvpgip4* appeared to be moderately induced by mechanical wounding. These results provide evidence that endopolygalacturonases contribute to the infection process during host colonization by promoting the release of plant cell oligogalacturonides, which are powerful signaling molecules and may also activate plant defenses, such as polygalacturonase-inhibiting proteins.

Key words: Endopolygalacturonase; *Phaseolus vulgaris*; White mold; Polygalacturonase-inhibiting protein; *Sclerotinia sclerotiorum*

INTRODUCTION

White mold, caused by the ascomycete *Sclerotinia sclerotiorum* (Lib.) de Bary, is a significant cause of yield loss in Brazilian dry bean (*Phaseolus vulgaris*) crops. This pathogen survives in the soil for several years as sclerotia and has a very broad host range of more than 400 species, including many yearly crops and weeds, thus enabling fungal populations to persist and spread easily (Boland and Hall, 1994). Effective and economically feasible disease control is laborious and dry bean cultivars are at present highly susceptible to the disease.

To infect and colonize the plant tissues, most fungal pathogens need to overcome the structural barrier represented by plant cell wall. Cell wall-degrading enzymes are deputed to this function, and those able to depolymerize the pectic component of the middle lamellae and primary cell walls are the most intensely investigated (Alghisi and Favaron, 1995; Annis and Goodwin, 1997). The contribution of cell wall-degrading enzymes to the pathogenicity or virulence of fungi has been studied in several pathosystems. Endopolygalacturonases (PGs) are among the first enzymes secreted during the infection process and cleave the α(1-4) linkages between D-galacturonic acid residues in homogalacturonan, causing cell separation and maceration of host tissue. The genome of *S. sclerotiorum* strain 1980 encodes five endo-PGs, four of which are expressed in culture or during infection (Li et al., 2004). The importance of polygalacturonases in plant diseases has been demonstrated for the pathogenic fungi *Botrytis cinerea* (ten Have et al., 1998), *Alternaria citri* (Isshiki et al., 2001), *Claviceps purpurea* (Oeser et al., 2002), and *S. sclerotiorum* (Kasza et al., 2004; Li et al., 2004).

Many plants possess a cell wall glycoprotein, polygalacturonase-inhibiting protein (PGIP), that is able to inhibit fungal PGs. The interaction between fungal PGs and plant PGIPs favors the accumulation of oligogalacturonides, which elicit a wide range of defense mechanisms (De Lorenzo et al., 2001; Ridley et al., 2001; De Lorenzo and Ferrari, 2002). A direct role for PGIPs in plant defense has been demonstrated recently where transgenic tomato and tobacco plants over-expressing PGIP showed a reduction in symptoms when infected by *B. cinerea* (Powell et al., 2000; Ferrari et al., 2003; Manfredini et al., 2005).

Recently, the development of expressed sequence tag resources, together with the

availability of high-quality genomic libraries and the progress in sequence analysis tools, have allowed complete or extensive characterization of the PGIP gene families in *Arabidopsis thaliana* (L.) Heynh. (Ferrari et al., 2003) and *Brassica napus* L. (Li et al., 2003; Hegedus et al., 2008). In *P. vulgaris*, four genes code for proteins that show sequence variation from 3 to 24%. These genes are grouped into two couples, *Pvpgip1-Pvpgip2* and *Pvpgip3-Pvpgip4*, probably originating from independent events of gene duplication (D'Ovidio et al., 2004).

In order to compare the temporal expression of *sspg* and *Pvpgip* genes, we performed semi-quantitative reverse transcription polymerase chain reaction (PCR) and real-time PCR experiments on total RNA extracted from dry bean stems at different times following inoculation with *S. sclerotiorum*. Simultaneous analysis of *sspg* and *Pvpgip* expression during plant infection could better elucidate and substantiate the role of these different components in plant-pathogen interaction.

MATERIAL AND METHODS

Infection and bean tissue sampling

S. sclerotiorum isolate SPS was collected from a naturally infected bean plant and grown on Petri dishes containing potato-dextrose agar culture medium for 5 days at 20°C. After this growth period, 1-cm long toothpick ends poked into culture medium were wrapped by the pathogen mycelia, which were used to inoculate dry bean plants afterwards. Plants of the dry bean cultivar Pérola were grown in a greenhouse at Embrapa Arroz e Feijão (Santo Antônio de Goiás, GO, Brazil), in 2-kg plastic pots with red yellow latosol soil that was treated with 5 g 4-30-16 NPK fertilizer. The inoculation was performed 10 days after emergence by introducing the toothpick ends wrapped with the pathogen mycelia into the base of the stems 1.0 cm above the soil surface. Plants that were mechanically wounded by introducing a toothpick without fungi into the base of their stems were used as an experimental control. All plants were kept at 20°C and 90% relative humidity for 48 h to provide adequate conditions for infection. Tissue samples were collected from both the necrotic part and the chlorotic area of each lesion within 2 cm of the lesion edges at 24, 48, 72, and 96 h post-inoculation (hpi) and immediately frozen in liquid nitrogen prior to RNA and protein extraction.

Semi-quantitative reverse transcription PCR

RNA was extracted from plant tissues and fungal material using the standard Trizol protocol (Gibco-BRL). To remove any genomic DNA contamination, RNA was treated with RNAse-free DNAse I (Promega) followed by enzyme inactivation (2.5 mM EDTA, 65°C/10 min) and ethanol precipitation.

The transcriptional analyses of fungal *pg* (*sspg*) and plant *pgip* (*Pvpgip*) genes were carried out employing semi-quantitative reverse transcription PCR and the following oligonucleotide primers specific for *pg* sequences: *sspg1* (AF501307) [pg1R: 5'-TCT TGC AGC AGT CGA GAA GA-3'; pg1F: 5'-GTG TTG TGT CCG AGG GAG TT-3']; *sspg3* (AY312510) [pg3R: 5'-ACC CAC CAC TTT GGC TAC TG-3'; pg3F: 5'-TGA GAC GGT AAG ACC CTT GG-3']; *sspg5* (AY496277) [pg5R: 5'-TGT CCA AGT TTT CAG TAT T-3'; pg5F: 5'-CTA CCA GCA TTT CCA TTA T-3'], and s*spg6* (AF501308) [pg6R: 5'-CAA GCT TAT TGG AAT

GGG TAT-3'; pg6F: 5'-CTG GAG TTG ACG ATT TGA CTA-3']. The sizes of all amplified fragments were 495, 448, 452, and 462 bp, respectively. The 28S rDNA gene-specific primers [rDNA-F (5'-GGT GGA GTG ATT TGT CTG-3') and rDNA-R (5'-CTT ACT AGG GAT TCC TCG-3')] were designed according to 28S rDNA gene of *S. sclerotiorum*. These pairs of primers were used to amplify a 600-bp cDNA product that acted as a control in the reverse transcription PCR experiments.

Transcripts of PGIP were amplified using specific primers for *Pvpgip1*, *Pvpgip2* (both *Pvpgip2.1* and *Pvpgip2.2*), *Pvpgip3*, and *Pvpgip4* as described by D'Ovidio et al. (2004).

First strand cDNA was synthesized using 2 µg DNAse-treated total RNA using superscript II Reverse Transcriptase (Gibco-BRL), following the supplier recommendations. We amplified 5 µL of first strand cDNA in a final reaction volume of 25 µL containing 1X Taq DNA polymerase buffer, 1.5 mM MgCl$_2$, 0.2 mM dNTPs, 0.2 µM specific primers, and 5 U Taq DNA polymerase (Gibco-BRL). PCR was initially performed at 94°C for 4 min, and then 27 cycles as follows: 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. PCR products were resolved by electrophoresis on a 1.2% w/v agarose gel. Two replicates of reverse transcription PCR experiments were performed for all genes analyzed. To quantify the gene expression levels, we employed densitometry analysis (Scion Image software) available online (http://www.scioncorp.com). Amplified product intensity was expressed as relative absorbance units. The ratio between the relative absorbance units determined for the amplified gene of interest and internal control was calculated to normalize for initial variations in sample concentration and as a control for reaction efficiency.

Real-time PCR

The sequences of primers used were: *Pvpgip1* (AJ864506.1) [pgip1 F - CGC CTC ACC GGG AAG AT; pgip1 R - TGT TCC GAG ACA AGT CAA CGA A]; *Pvpgip2* (AJ864507.1) [pgip2 F - TTC GAC GGC AAC CGA ATC; pgip2 R - TGG TCA TCG ACG TAA ACA GCT T]; *Pvpgip3* (AJ864508.1) [pgip3 F - TCC TTC CCG AAG CAT TTC AC; pgip3 R - GCG TCG CCG GTA TAT TGC]; *Pvpgip4* (AJ864509.1) [pgip4 F - TCC TTC CCG AAG CAT TTC AC; pgip4 R - GCC AGC GTC GTC GGA ATA T], and actin gene (*act*) of *P. vulgaris* (Q11197) [act-F - 5' CCC CAG CGT TCT ACG TCT 3', act-R - 5' CAT GTC AAC ACG AGC AAT G 3']. The sizes of all amplified fragments were 70, 76, 84, 106, and 92 bp for *Pvpgip1-4* and actin genes, respectively.

Equal amounts of RNA (0.5 µg) were reverse-transcribed using oligo(dT)12-18 primer and submitted to real-time PCR. Amplification assays were carried out with a 7900HT Sequence Detection System ABI PRISM instrument (Applied Biosystems, USA) in 12-µL reaction mixtures containing 0.4 µM of each primer described above, 6 µL SYBR Green PCR Master mix (2X), and 0.2 µL template cDNA. After an initial denaturation step at 95°C for 10 min, amplifications were carried out for 40 cycles at 95°C for 15 s and 60°C for 1 min. To check the specificity of the PCR product, melting curves were analyzed for each data point. Relative gene expression was obtained with the formula: fold induction = $2^{-[\Delta\Delta Ct]}$, where $\Delta\Delta Ct$ = [Ct GI (unknown sample) - Ct ACT (unknown sample)] - [Ct GI (reference sample) - Ct ACT (reference sample)]. GI is the gene of interest and ACT is the *P. vulgaris* actin gene used as internal control. The reference sample was chosen to represent 1X expression of the gene of interest.

Enzyme assay

Polygalacturonase activity was quantified by recording the increase in absorbance at 575 nm caused by the release of reducing sugars from citrus pectin. The reaction mixture contained 25 μL macerated tissue extract and 225 μL 2 mg/mL citrus pectin dissolved in 0.1 M sodium acetate buffer, pH 5. After 30 min incubation at 45°C, the reaction was stopped by the addition of 750 μL dinitrosalicylic acid reagent, boiled for 5 min, and cooled on ice (Miller, 1959). Enzyme and substrate controls were included in all assays. Enzyme activities are expressed as micrograms of reducing sugar released during 30-min incubation. Protein contents were determined using the Quant-iT Protein Assay kit (Invitrogen).

RESULTS AND DISCUSSION

Aiming at comparing the temporal expression of *sspg* and *Pvpgip* genes, semi-quantitative real-time PCR experiments were performed on total RNA extracted from bean stem lesions at different times after inoculation with *S. sclerotiorum*. These experiments were carried out employing specific primers directed to the internal control (rDNA 28S) and to the experimental *sspg* (*sspg1*, *sspg3*, *sspg5*, *sspg6*) and *Pvpgip* (*Pvpgip1*, *Pvpgip2*, *Pvpgip3*, *Pvpgip4*) genes. Afterward, we performed reverse transcription PCR product quantification using the appropriate analyses, which revealed that both *sspg* and *Pvpgip* genes showed high expression levels as a consequence of *S. sclerotiorum* infection.
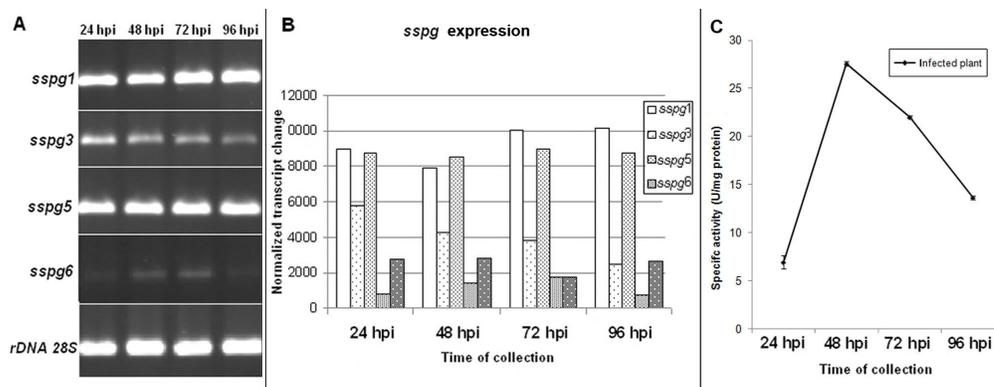
Expression of *S. sclerotiorum PG* genes during pathogenesis

Dry bean plants were effectively infected by *S. sclerotiorum* (Figure 1). During the first 24 hpi, a mycelial mass developed and invaded the tissues, but no macroscopic symptoms were detectable. At 48 hpi, a necrotic zone was observed around the inoculation site and at 72 hpi, abundant aerial mycelia emerged from plant decayed tissues, which showed maceration on the surface. From 96 hpi on, the mycelium became progressively less abundant while the plant tissues were totally macerated.
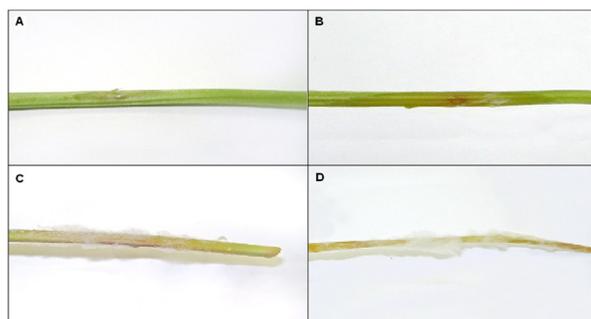
Figure 1. *Sclerotinia sclerotiorum*-infected bean stem at different times after inoculation. Inoculation performed 10 days after emergence, by introducing mycelium-colonized toothpick ends into the base of the stems 1.0 cm above the soil surface, which were removed to take the photograph in order to show the lesions at the following times: **A.** 24 hpi. **B.** 48 hpi. **C.** 72 hpi. **D.** 96 hpi. Tissue samples collected within 2 cm of the lesion margin.

The expression of *sspg* genes was assessed in infected tissues to determine whether any of them could be associated with the infection process. Transcripts of the *sspg1*, *sspg3*, *sspg5*, and *sspg6* genes were detected by semi-quantitative reverse transcription PCR at 24 hpi (Figure 2A,B). At this point, a mycelium developed and invaded the tissues, but no macroscopic symptoms were detectable.

Figure 2. Expression pattern of polygalacturonase genes (*sspg1*, *sspg3*, *sspg5*, *sspg6*) of *Sclerotinia sclerotiorum* during pathogenesis of bean stems. **A.** Ethidium bromide-stained reverse transcription polymerase chain reaction (PCR) products: Tissue samples were collected from the necrotic part of each lesion 24, 48, 72, and 96 hpi. Total RNA was extracted and used in reverse transcription PCR experiments as described in the Material and Methods section. A fragment of rDNA was used to evaluate the presence of this fungus in the affected tissues. **B.** The quantitative analysis of *sspg* genes expression was performed by densitometry employing the Scion Image software (http://www.scioncorp.com). The *sspg* transcript levels were calculated in relation to the internal control. **C.** Endopolygalacturonase activity during pathogenesis of bean stems (see Material and Methods section).

The most significantly induced gene during the early phases of infection was *sspg1*, and the level of its transcript increased strongly at 96 hpi, when the stems were completely invaded by mycelia. Recent evidence suggests that *sspg1* plays a role both in the initiation of the infection and in the subsequent lesion expansion during interaction of *S. sclerotiorum* with *B. napus* (Cessna et al., 2000; Li et al., 2004). In *B. cinerea*, the systematic appearance of individual PGs attests to the role that each plays in the infection process: *Bcpg1* and *Bcpg2* are expressed in the earliest stages of infection (ten Have et al., 2001), and mutants unable to produce BCPG1 exhibit reduced rates of lesion expansion (ten Have et al., 1998).

In our study, the *sspg3* gene was expressed during the initial phase of colonization of healthy plant tissues and peaked at 24 hpi, when the fungal biomass was very low. Its expression decreased at later stages of host colonization. This pattern of expression suggests that *sspg3* may be more implicated in the early stages of host colonization than during the late stages of maceration of host tissues; *sspg5* was expressed at a high level 24 hpi and the transcripts accumulated between 72 and 96 hpi, the phase of invasive growth of the pathogen and progression of the necrotic zone; *sspg6* was regulated during the final phase of maceration and its transcript reached a maximum at 72 hpi, and then slightly decreased when the host tissues were macerated (96 hpi). A fragment of fungal 28S rRNA was amplified as a control for the amount of fungal RNA present in each sample.

The expression timeframe of *sspg* genes was similar to those reported for *pgs* of other *S. sclerotiorum* isolates (Cotton et al., 2002; Kasza et al., 2004; Li et al., 2004), although the respective patterns were different. For example, the expression of *pg1* precedes that of *pg3*, *pg5*, and *pg6* during infection of carrot roots with *S. sclerotiorum* (Kasza et al., 2004). In that pathosystem, *pg1-3* genes were expressed during the phase of colonization of healthy plant tissues, whereas *pg5* was transcribed during the final phase of maceration, and *pg6* and *pg7* exhibited a more constant expression pattern (Kasza et al., 2004). Studying *B. napus* leaves infected with *S. sclerotiorum*, Li et al. (2004) reported that *sspg1* gene was highly expressed during infection; *sspg3* was also expressed during the early phase of infection, although lower less than *sspg1*, whereas *sspg5* and *sspg6* were only weakly expressed. Curiously, our study indicates that *sspg5* gene was highly expressed during the infection process.

Examination of the expression patterns of individual *sspg* genes has revealed that the interplay of PGs among themselves and with the host during the various stages of infection is finely coordinated. Early in pathogenesis, *S. sclerotiorum* secretes oxalic acid in infected tissues, which gradually lowers the neutral pH of the plant apoplasm. In the course of infection, acidification of the ambient medium and decay of the plant tissue polymers may create an environment conducive for early or constitutive PGs to attack the host cell wall, in turn releasing metabolites that serve to induce expression of other *sspg* genes (Fraissinet-Tachet and Févre, 1996; ten Have et al., 2001).

We also investigated whether the presence of multiple genes could respond to PG activity modulation. During the pathogenesis process in infected tissue, PG activity increased and peaked at approximately 48 h, and then gradually decreased at the times evaluated (Figure 2C).

In addition to previous reports (Kasza et al., 2004; Li et al., 2004), our data indicate that *sspg* genes are differentially regulated under parasitic conditions, the expression of each of them being governed by a unique set of environmental and developmental factors. However, the specific role individual PG plays in the infection process remains to be elucidated.

Members of the *Pvpgip* family are differentially regulated

In order to verify whether the four *Pvpgip* genes display different regulation patterns following wounding and pathogen inoculation, we performed a semi-quantitative reverse transcription PCR analysis using specific primers for each gene. Transcript level analyses were performed on total RNA extracted from 1- to 4-day-old bean seedlings mechanically wounded or infected with *S. sclerotiorum*.

Even with a high number of amplification cycles and at an RNA template concentration higher than that used for the analysis of *Pvpgip* genes of infected samples, amplification of *Pvpgip1-3* never occurred in RNA samples extracted from mechanically wounded plant tissues. Remarkably, only *Pvpgip4* transcripts appeared to be induced by mechanical wounding (Figure 3A).

Densitometric analyses revealed that *Pvpgip* transcripts were differentially induced in dry bean stem tissue in response to *S. sclerotiorum* infection (Figure 3B). During the early phases of infection, *Pvpgip1* transcripts were not detected, whereas their presence was revealed at 72-96 hpi, when the stems were completely invaded by mycelia. *Pvpgip2* levels were high in the initial phase of colonization and peaked at 48 hpi. The levels of transcript declined between 72 and 96 hpi as the plant tissue began to undergo necrosis.
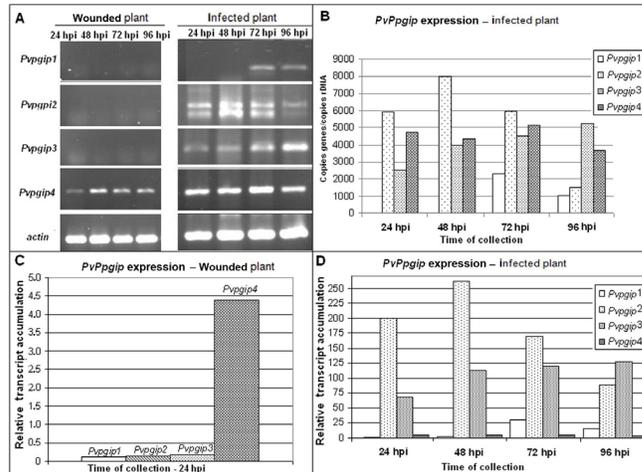
Figure 3. Differential expression of dry bean *Pvpgip* genes in response to infection with *Sclerotinia sclerotiorum*. **A.** Total RNA was extracted from bean stem at different times (24, 48, 72, and 96 h) after wounding (wounded plant) or infection with *S. sclerotiorum* (infected plant). Reverse transcription polymerase chain reaction (PCR) experiments were carried out as described in the Material and Methods section. The sizes of the amplified DNA fragments are as follows: *Pvpgip1* = 493 bp; *Pvpgip2* = 443 bp; *Pvpgip3* = 451 bp; *Pvpgip4* = 445 bp. *rDNA* 28S gene was used to control the amount of mRNA present in each sample. **B.** Semi-quantitative analyses of *Pvpgip* expression in infected tissue were performed exactly as described in Figure 2. **C.** and **D.** Transcript accumulation of *Pvpgip* genes in wounded or infected plants. Real-time PCR analyses were performed and transcript levels were calculated from triplicate data using the standard curve method: **C.** *P. vulgaris* actin gene as internal control and health plant (at time zero) as reference sample; **D.** *P. vulgaris* actin gene as internal control and wounded plant (at respective time) as reference sample. The results represent the mean fold increase of mRNA level over wounded stem, plotted against the 1X expression level. Results are reported as means ± standard deviation of three experiments.

Pvpgip3 expression increased strongly at 96 hpi. A reverse transcription PCR product derived from transcripts of *Pvpgip4* was present from 24 hpi until the end of the experiment, but its expression was detected at higher levels in the necrotic lesion area than in the mechanically wounded plant.

These expression data clearly characterize a sequential pattern of gene transcription and suggest that *Pvpgip2* may be more involved in the early stages of host defense and *Pvpgip1* and *Pvpgip3*, during the late stages. This profile expression was confirmed by real-time PCR analyses.

Pvpgip genes are regulated during development and upon wounding and pathogen infection or treatments with eliciting agents such as salicylic acid and cold (De Lorenzo et al., 2001; Ferrari et al., 2003). Analyses performed by D'Ovidio et al. (2004) resulted in the identification of four *Pvpgip* in *P. vulgaris*. Furthermore, individual *Pvpgip* are differentially expressed in response to pathogen infection or treatments with elicitors: the expression of *Pvpgip3* is induced by oligogalacturonides but not by fungal glucan, salicylic acid, or wounding; on the other hand, expression of *Pvpgip4* is not altered by any of these treatments.

Expression of *Pvpgip1* is induced by wounding only, whereas *Pvpgip2* is upregulated by oligogalacturonides, salicylic acid, and wounding. However, except for the amplification of *Pvpgip1*, which never occurred in RNA samples extracted at time 0 from either untreated tissues or suspension cultured cells, basal levels of transcripts of *Pvpgip1*, *Pvpgip2*, *Pvpgip3*,

and *Pvpgip4* were detected by reverse transcription PCR in all RNA samples extracted from bean hypocotyls at different times after wounding (D'Ovidio et al., 2004).

In contrast, we report here that transcripts of *Pvpgip1*, *Pvpgip2*, and *Pvpgip3* were not detected by semi-quantitative reverse transcription PCR in bean stem upon wounding. In order to confirm their regulation, real-time PCR approach was used. Primers specific for *Pvpgip1*, *Pvpgip2*, *Pvpgip3*, and *Pvpgip4* were synthesized (see Material and Methods section).

Real-time PCR analyses revealed very low basal levels of *Pvpgip1*, *Pvpgip2*, and *Pvpgip3* genes in *P. vulgaris* stems 24 h after wounding (Figure 3C). The values represent a higher number of transcripts in relation to the healthy plants used as reference in these analyses. Such discrepancies between data attained on bean hypocotyls (D'Ovidio et al., 2004) and those obtained on stem, however, are not surprising because additional factors occurring in plant tissue may condition PG activity or PG-PGIP interaction. Although other factors such as cultivar, phase of plant development, and cultivation conditions may have influenced the expression of *Pvpgip*, further studies are necessary to prove this.

In infected bean stem, the expression of *Pvpgip1*, *Pvpgip2*, *Pvpgip3*, and *Pvpgip4* genes was rapidly and transiently upregulated (Figure 3D). Compared to the expression in injured plants, induction of *Pvpgip* genes during stem infection by *S. sclerotiorum* peaked at: 48 hpi for *Pvpgip2*, showing a 262-fold increase in the amount of transcripts; 96 hpi for *Pvpgip3*, with a 112-fold increase; 72 hpi for *Pvpgip1*, with a 27.2-fold increase, and *Pvpgip4* showing an average of 4.9-fold increase at the times evaluated. Transcript accumulation of these genes slowly decreased until 96 hpi. Similar expression profiles were previously observed by semi-quantitative reverse transcription PCR.

Remarkably, *Pvpgip2* gene, which encodes the most efficient inhibitor of fungal PGs characterized so far (D'Ovidio et al., 2004; Manfredini et al., 2005), was the family member most upregulated by infection stimuli. *Pvpgip2* has been previously found to have different types of inhibition, which suggests that the inhibitor adapts the recognition capabilities of its wide concave surface in many ways and against different epitopes of different PG ligands (Sicilia et al., 2005).

Plant defenses against fungal pathogens consist of both a localized response that is often associated with an oxidative burst and a more generalized systemic response mediated by signaling molecules. PGIPs have been shown to halt fungal invasion in plants. Following inoculation with *B. cinerea*, transgenic tomato and grapevine plants overexpressing a *pgip* gene from pear (Powell et al., 2000; Agüero et al., 2005), *Arabidopsis* plants overexpressing two endogenous *pgip* genes (Ferrari et al., 2003), and tobacco plants overexpressing bean *Pvpgip2* (Manfredini et al., 2005) developed smaller lesions than those in wild-type plants, suggesting that PGIPs play a role in the innate immunity of plants and contribute to their basal resistance against fungi.

The PG-PGIP interaction limits the aggressive potential of PGs and favors the accumulation of elicitor-active oligogalacturonides in the apoplast (De Lorenzo and Ferrari, 2002). Oligogalacturonides are general elicitors of a wide number of plant defenses, including accumulation of phytoalexins, synthesis of lignin, expression of β-1,3-glucanase and proteinase inhibitors, and production of reactive oxygen species (Ridley et al., 2001; De Lorenzo and Ferrari, 2002). PGs are also capable of initiating programmed cell death in the host leading to tissue necrosis, a phenomenon that is circumvented by PGIPs (Zuppini et al., 2005). The capacity of PGs to elicit defense responses unrelated to their enzymatic activity has been recently proposed for *B. cinerea*-grapevine interaction (Poinssot et al., 2003). However,

the enzymatic activity of PG from *Colletrotrichum lindemuthianum* has been shown to be essential for activating protective responses in tobacco (Boudart et al., 2003).

We showed different temporal expression of *pg* and *Pvpgip* genes in the course of bean stem infection. Our results seem to be consistent with a maximization of fungal PG activity in the host tissue. By promoting the release of plant cell wall polysaccharide fragments and releasing oligogalacturonides, which are powerful signaling molecules, this enzyme may also activate plant defenses such as the increased expression of polygalacturonase inhibitor proteins. Future studies will aim at identifying cell surface proteins specifically induced during the first steps of pathogenesis by a proteomic approach. This should help unravel the complex developmental program underlying the infection process. In order to improve plant resistance through a PGIP-based strategy, knowledge of specific relationships between PGs and PGIPs is crucial.

ACKNOWLEDGMENTS

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