Characterization of Two Closely Linked Soybean PGIP Genes and Transcript Regulation Following Pathogen Infection and Wounding

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Abstract

Polygalacturonase-inhibiting protein (PGIP) is a plant cell wall protein that regulates the action of fungal endopolygalacturonases (PG) favouring the formation of oligogalacturonides active as elicitors of plant defense responses. We have isolated two novel soybean PGIP genes (*Gmpgip1* and *Gmpgip2*) and their recognition specificities against fungal PGs have been tested by expressing them in *Nicotiana benthamiana* using the *Potato virus X* (PVX) as vector and in *Escherichia coli*. In both systems GmPGIP1 and GmPGIP2 accumulate but showed no activity against PGs. Transcript regulation of GmPGIP1 and GmPGIP2 showed that these genes undergo a differential regulation following *Sclerotinia sclerotiorum* infection and wounding.

Keywords: *Sclerotinia sclerotiorum*; *Glycine max*; PGIP; polygalacturonase; gene family; transcript regulation

INTRODUCTION

Polygalacturonase-inhibiting proteins (PGIPs) are leucine-rich repeat (LRR) glycoproteins associated with the cell wall of both monocots and dicots plant species. Due to their ability to recognize and inhibit endopolygalacturonases (PGs) from phytopathogenic fungi, they are typical defence proteins induced by pathogen infection and stress-related signals (DE LORENZO *et al.* 2001). PGIP may therefore represent an important defence mechanism against those pathogens producing PG during tissue colonization. At this regards, a reduction of symptoms caused by the growth of the fungal pathogen *Botrytis cinerea* have been obtained in transgenic tomato plant overexpressing a pear *pgip* gene (POWELL *et al.* 2000).

Genes encoding PGIPs are organized into families whose members can encode for proteins showing different recognition specificities against fungal PGs. As consequence, knowledge of PGIP specificities contained into a single genotype is an essential step to identify both the specific contribution to PG inhibition and to ascertain possible additional physiological roles.

Molecular hybridization analysis has shown that the soybean *pgip* gene family is composed by a small number of members and so far two different incomplete genes, isolated by PCR, have been reported (FAVARON *et al.* 1994; MAHALINGAM *et al.* 1999). Here we report two novel soybean *pgip* genes isolated from a genomic library and describe preliminary results on their regulation following wounding and pathogen infection.

MATERIALS AND METHODS

A lambda phage library from *Glycine max* cv. Williams 82 was screened with the soybean pGM7 probe (FAVARON *et al.* 1994). The *Escherichia coli*

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pET system (Novagen) was used to express the mature coding region of soybean *pgip* genes. Purification and refolding of the heterologously expressed proteins were performed using the "Protein refolding Kit" (Novagen). Heterologous expression in *Nicotiana benthamiana* using as vector the potato virus X (PVX) (BAULCOMBE *et al*. 1995) was performed as previously described (LECKIE *et al*. 1999). Fungal endo-PG purification and PG and PGIP assays were peformed as previously reported (FAVARON *et al*. 1994). Soybean hypocotyls were infected with plugs of *S. sclerotiorum* mycelium. RNA extractions were performed with the "Nucleo Spin RNA Plant" kit (Macherey-Nagel) and RT-PCR was performed using the Ready-To-Go RT-PCR beads (Amersham Pharmacia Biotech).

RESULTS AND DISCUSSION

A genomic library from *Glycine max* cultivar Williams 82 was screened with the pGM7 probe (FAVARON *et al*. 1994) and a clone containing two different *pgip* genes, named *Gmpgip1* and *Gmpgip2*, was isolated. Nucleotide sequence analysis showed that these two genes share 80% of similarity and encode for PGIPs with the characteristic LRR structure. These genes represent the first complete soybean *pgip* genes so far described since the previous reported pGM7 (FAVARON *et al*. 1994) and GmPGIP (MAHALINGAM *et al*. 1999) are both lacking of the leader sequence and their terminal regions correspond to the heterologous primers used in the PCR cloning procedure.

In order to analyze the individual contribution of GmPGIP1 and GmPGIP2 to the bulk PGIP activity obtained from soybean tissue, we have cloned the mature coding region of *Gmpgip1* and *Gmpgip2* into the bacterial pET3a expression vector. Both GmPGIP1 and GmPGIP2 accumulate in good quantity in protein bodies but, after refolding, they show no activity against fungal endo-PG (Figure 1). As control sample, the mature coding region of Pvpgip2 from bean, with known inhibition activities (LECKIE *et al*. 1999), was also expressed in *E. coli* but none inhibition activity was obtained. This lack of activity could be due to an incorrect folding or to the lacking of glycosilation. To overcome this difficulties we have expressed the complete coding region of *Gmpgip1* and *Gmpgip2* in *Nicotiana benthamiana* using the PVX expression cassette (BAULCOMBE *et al*. 1995), since it was previously demonstrated the effectiveness of this system in producing active PGIP (LECKIE *et al*. 1999). However, inhibition assays performed against some PGs showed that even in this case the heterologous proteins were expressed but none inhibition activity was observed.

To study whether *pgip* genes are differentially regulated during pathogen infection and wounding

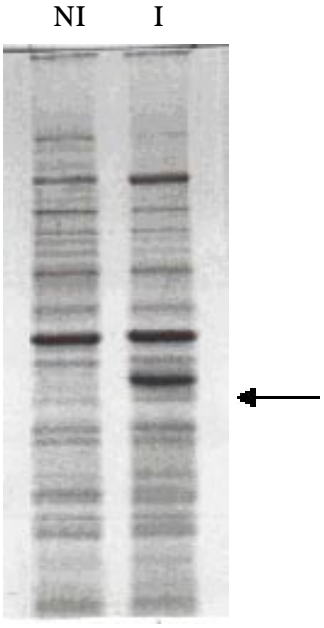

Figure 1. SDS-PAGE of the heterologous GmPGIP1 expressed in *E. coli*. Analysis were carried out using 10 μl aliquotes from non induced (NI) and IPTG induced (I) *E. coli* culture. Arrow indicates the induced GmPGIP1

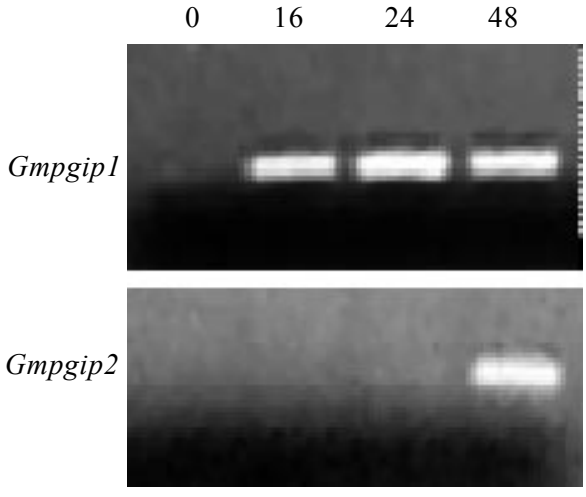

Figure 2. 1.5% agarose gel of RT-PCR products from soybean hypocotyls infected with *S. sclerotiorum*. 0, 16, 24, 48, indicate hours after mycelium inoculation

we have performed RT-PCR analyses with primers specific for *Gmpgip1* and *Gmpgip2*. These analyses were carried out on RNA extracted from soybean hypocotyls at different times after wounding or infection with *Sclerotinia sclerotiorum*. *Gmpgip1* is up regulated following both wounding and infection, whereas *Gmpgip2* is not induced by wounding and is tardively up regulated after infection with *S. sclerotiorum* (Figure 2).

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