Physiological and Molecular Plant Pathology 102 (2018) 200–208

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp

Sugar beet polygalacturonase-inhibiting proteins with 11 LRRs confer *Rhizoctonia*, *Fusarium* and *Botrytis* resistance in *Nicotiana* plants

Haiyan Li, Ann C. Smigocki*

USDA-ARS, Molecular Plant Pathology Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705, USA

ARTICLE INFO

Keywords:
Sugar beet
PGIP
LRR
Rhizoctonia
Fusarium
Botrytis

ABSTRACT

Polygalacturonase-inhibiting proteins (PGIPs) are leucine-rich repeat (LRR) proteins that inhibit polygalacturonase (PG) enzymes secreted by pathogens to break down plant cell walls during early stage of disease development. Sugar beet (*Beta vulgaris* L.) PGIP genes (*BvPGIPs*) have 11 LRR domains as compared to 10 LRRs generally found in other plant species. To determine whether the *BvPGIPs* have a function in plant defense, genes encoding BvPGIP1 and BvPGIP2 that differ in 8 amino acids were fused with the CaMV 35S constitutive promoter and introduced into *Nicotiana benthamiana*. Crude PGIP protein extracts from *BvPGIP1* transgenic plants significantly inhibited *Rhizoctonia solani*, *Fusarium solani* and *Botrytis cinerea* PGs. BvPGIP2 extract also inhibited PGs from *F. solani* and *B. cinerea* but did not inhibit PGs from *R. solani*. When transgenic *BvPGIP* plants were bioassayed for resistance, similar results were obtained. This is a first report that documents sugar beet PGIPs with 11 LRRs confer resistance to three different fungal pathogens.

1. Introduction

Microbial pathogens produce a series of hydrolytic enzymes to initiate disease onset in plants. Polygalacturonases (PGs) are among the enzymes that have been shown to break down the polygalacturonate chain in plant cell walls. To defend themselves, plants are known to produce polygalacturonase-inhibiting proteins (PGIPs) to inhibit the action of the PGs. Plant PGIPs are extracytoplasmic cell wall proteins that typically contain 10 imperfect leucine-rich repeats (LRR; LxxLxLxxNxLT/SGxIPxxLxxLxx) [5,13]. However, PGIPs with fewer or more than 10 LRR domains have been reported. Rice OsPGIP1, alfalfa MtPGIP2 and wheat TaPGIP3 all contain 9 LRR domains [11,12,27] whereas 11 LRRs were reported in sugar beet BvPGIPs [19,20].

The involvement of PGIPs in defense mechanisms was demonstrated in many plants. Cloned *PGIP* genes that were expressed in transgenic plants were shown to alter the cell wall structure in uninfected plants [32] and reduced disease symptoms induced by fungal and bacterial pathogens [17]. Control of fungal diseases caused by *Alternaria solani*, *Bipolaris sorokiniana*, *Botrytis cinerea*, *Fusarium graminearum*, *F. oxysporum vasinfectum*, *Rhizoctonia solani* and many other fungal pathogens was reported with recombinant PGIPs [2,4,8,11,15,21–24,29]. Tobacco transformed with the grapevine *VvPGIP1* or bean *PvPGIP2* showed significantly reduced susceptibility to *B. cinerea* [15,23], but only *PvPGIP2* was also able to increase resistance to *R. solani* AG3 [2]. PGIP are generally highly specific and selective for PG targets, and a variation of even a single amino acid in the xxLxLxx motif often confers a new PG recognition capacity [6,18]. This explains the observed differences in the inhibitory abilities associated with species-specific PGIPs or within the same species. For example, the 4 bean PGIPs (PvPGIP1, PvPGIP2, PvPGIP3, PvPGIP4) have varied specificities against several different fungal PGs, but only PvPGIP3 and PvPGIP4 were shown to inhibit PGs of mirid insects [6]. Eight amino acids differences, five of them confined within the xxLxLxx motif, were found in PvPGIP1 and PvPGIP2, conveying specificity of PvPGIP1 for *Aspergillus niger* not *F. moniliforme* PG, unlike PvPGIP2 that inhibits both PGs [4,18]. Differential expression of 4 soybean PGIP genes (*GmPGIPs*) was reported after plants were wounded or infected with *Sclerotinia sclerotiorum* [16]. In sugar beet, BvPGIP protein extracts prepared from a root maggot resistant F1016 germplasm inhibited PGs of both *R. solani* and *F. oxysporum*; however, PGIPs from a susceptible F1010 variety only inhibited *F. oxysporum* PG [20]. The difference in the *R. solani* response suggests that there are distinct PGIP repertoires within the 2 sugar beet lines with unique specificities for fungal PGs.

In this study, we characterized the defense role of two sugar beet BvPGIPs with 11 LRR domains. Two highly homologous *BvPGIP* genes, *BvPGIP1* and *BvPGIP2* that differ in 8 amino acids localized primarily in the LRR domains, were engineered for over-expression in *N. benthamiana* to determine if the small variations between BvPGIP1 and BvPGIP2 would provide distinguishable inhibitory activity towards fungi. Recombinant BvPGIPs prepared from transgenic plants carrying

* Corresponding author.
E-mail address: ann.smigocki@ars.usda.gov (A.C. Smigocki).

https://doi.org/10.1016/j.pmpp.2018.03.001
Received 16 February 2018; Received in revised form 9 March 2018; Accepted 14 March 2018
Available online 15 March 2018
0885-5765/ © 2018 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

BvPGIP1 or *BvPGIP2* were tested against fungal PGs purified from *R. solani*, *F. solani* and *B. cinerea*. Whole plants were also bioassayed for resistance to the 3 fungal pathogens. We report on the distinct patterns of inhibition observed with *BvPGIP1* and *BvPGIP2* plants.

2. Materials and methods

2.1. Plant and fungi

Nicotiana benthamiana Domin was used for plant transformation. Seeds were germinated in soil at room temperature. Seedlings were grown in the growth chamber at 24 °C with a 16 h photoperiod. Two-3 months old plants were maintained in the green house under similar conditions.

Two isolates of each of 3 pathogenic fungal strains were used, *R. solani* AG2-2 R1 and AG4 Rzc27, *F. solani* F03-78 and F04-17 and *B. cinerea* B009-1 and B009-8, all of which originated from sugar beet except F03-78 (Linda Hanson, USDA, ARS, East Lansing, MI). Fungi were grown on potato dextrose agar (PDA) media (BD Difco™, USA) for 7–10 days at room temperature.

2.2. Plant transformation with BvPGIP1 or BvPGIP2

The full-length coding sequences of *BvPGIP1* and *BvPGIP2* were cloned as previously described [20]. *BvPGIP1* and *BvPGIP2* were introduced separately into Gateway vectors (Gateway Technology Clonase II Manual; Invitrogen, Carlsbad, CA) and transferred into plant transformation vector pH7WG2D.1 (http://gateway.psb.ugent.be/) that contains the cauliflower mosaic virus (CaMV 35S) promoter and the hygromycin selectable marker gene (*hyg*) (Fig. 1A). The resulting transformation vectors were then introduced into *Agrobacterium tumefaciens* strain EHA105.

N. benthamiana leaf disks were used for transformation as previously described [26]. Briefly, the *A. tumefaciens* EHA 105 strain harboring either *BvPGIP1* or *BvPGIP2* was co-cultivated with *N. benthamiana* leaf explants (1 cm^2) that were excised from fully expanded leaves of greenhouse-grown plants. Sterilized explants were immersed in bacterial suspensions for 10 min, blotted dry on sterile filter paper and placed on MSB5 media (Murashige and Skoog basal salt and B5 vitamins, PhytoTechnology Laboratories, Overland Park, KS). After 2 days of co-cultivation in the dark at 25 °C, explants were washed with sterile solutions of cefotaxime and carbenicillin (500 mg/l each) and placed on callus-induction medium (CIM: MS salts, B5 vitamins, 6-benzylaminopurine (BAP) 2 mg/l, 200 mg/l cefotaxime and 500 mg/l carbenicillin). Regenerated shoots were excised and cultured on 1/2 B5 selection medium (SM) containing BAP 0.5 mg/l and Hyg 20 mg/l. Shoots were transferred to rooting medium (RM: 1/2 B5 medium with no hormones, supplemented with Hyg 20 mg/l). Rooted shoots were acclimated, transferred to a growth chamber (25 ± 2 °C day, 22 ± 2 °C night and 16/8 h light conditions, respectively) and then to a greenhouse (25 ± 5 °C day, 22 ± 3 °C night, 16/8 h light conditions). Leaf disks were also transformed with an empty vector as a negative control. Seeds collected from the regenerated plants (T0) were germinated on 1/2 MS salts with Hyg (40 mg/l) to select Hyg resistant T1 plants that segregated at a 3:1 ratio of resistant to susceptible plants.

2.3. Southern blot

Genomic DNA isolation and Southern blot analysis were performed by following the procedures as previously described [20] with modifications. Briefly, approximately 20 µg of genomic DNA was digested with 2 different restriction enzymes, *Xba*I and *Nco*I or *Xba*I and *Nhe*I (New England BioLabs Inc., Ipswich, MA), to detect *BvPGIP1* or *BvPGIP2* in transgenic plants. These enzymes do not cut within the *PGIP* genes. The full-length cDNA sequence was used as probe to detect each *PGIP* gene. Probes were detected through alkaline phosphatase color reaction following the instruction of DIG High Prime DNA Labeling and Detection Starter Kit II (Roche, Branchburg, NJ). The membrane was incubated in NBT/BCIP (1:50 dilution) solution (Sigma) at 30 °C in the dark for 2 h or until clear signals appeared. Signals were photographed with a Nikon D70 camera under natural light.

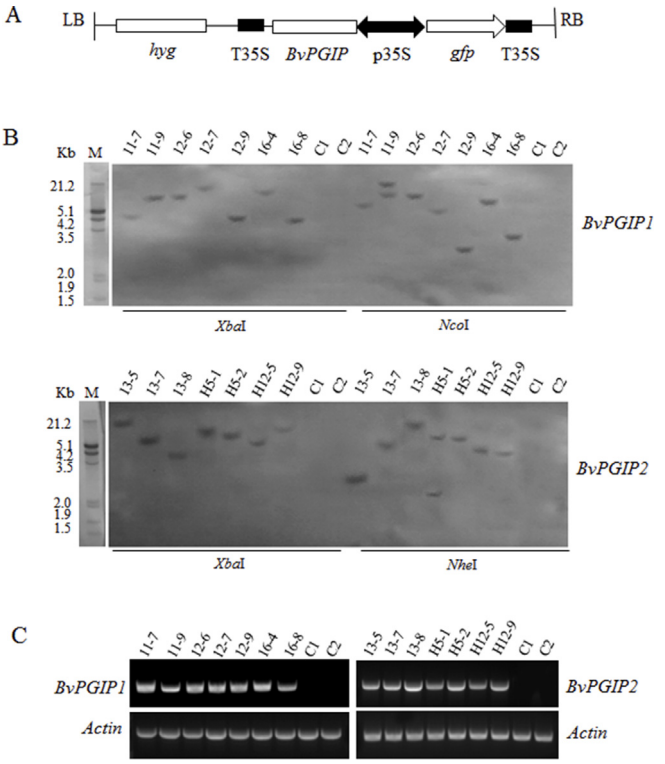

Fig. 1. Characterization of recombinant *BvPGIP* genes in *N. benthamiana* plants. (A) Schematic of reconstructed *BvPGIPs* in pH7WG2D.1 transformation vector. LB. left border; RB. right border; *Hyg*. hygromycin selectable marker gene; p35S. CaMV 35S promoter; T35S. CaMV 35S terminator; *gfp*. the green fluorescent protein reporter gene. (B) Southern blot analysis of independently-derived T2 homozygous plants of *BvPGIP1* (11-7, 11-9, 12-6, 12-7, 12-9, 16-4, 16-8) and *BvPGIP2* (13-5, 13-7, 13-8, H5-1, H5-2, H12-5, H12-9). Genomic DNA (20 µg) was digested with 2 different restriction enzymes (*Xba*I, *Nco*I- *BvPGIP1*; *Xba*I, *Nhe*I- *BvPGIP2*) that do not cut within the *BvPGIP* genes, and probed with the full-length cDNA fragment of each gene. (C) Over-expression of *BvPGIPs* in *N. benthamiana* plants. RT-PCR was performed with total RNA using gene-specific primers that amplify about 1.03 Kb of the coding region of *BvPGIP1* and *BvPGIP2*. The tobacco *actin* gene was used as an internal control. C1 represents the normal, untransformed tobacco plant. C2 represents the empty vector control plant.

2.4. RT-PCR

Total RNA was isolated from 100 mg of tobacco leaves using RNeasy Plant Mini Kit and treated with RNase-free DNase (Qiagen, Germantown, MD). As previously described, a partial coding region (1.03 Kb) of *BvPGIP1* or *BvPGIP2* was amplified using gene specific primers (PGIP1F/1R for *BvPGIP1* and PGIP2F/2R for *BvPGIP2*) [20]. The tobacco *actin* gene primers (Forward 5′-GCCACACTGTCCCCATC TAT- 3′ and reverse 5′-AACCACCTTGACCTTCATGC- 3′) were utilized to amplify a 512-bp fragment serving as an internal control to normalize the RT-PCR results. Gene expression was quantified by densitometry with an AlphaImager HP (Alpha Innotech, San Leandro, CA). RT-PCR analyses were repeated 2 times with comparable results.

2.5. Preparation of BvPGIP and fungal PG extracts

PGIP protein extracts were prepared from 5 g of tobacco leaves and

Download English Version:

https://daneshyari.com/en/article/8649253

Download Persian Version:

https://daneshyari.com/article/8649253

Daneshyari.com