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Research article

Different roles of glycine-rich RNA-binding protein7 in plant defense against *Pectobacterium carotovorum*, *Botrytis cinerea*, and tobacco mosaic viruses

Hwa Jung Lee, Jin Seo Kim, Seung Jin Yoo, Eun Young Kang, Song Hee Han, Kwang-Yeol Yang, Young Cheol Kim, Brian McSpadden Gardener, Hunseung Kang*

Department of Plant Biotechnology and Kumho Life Science Laboratory, College of Agriculture and Life Sciences, Chonnam National University, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, Republic of Korea

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ABSTRACT

Glycine-rich RNA-binding protein7 (AtGRP7) has previously been demonstrated to confer plant defense against *Pseudomonas syringae* DC3000. Here, we show that AtGRP7 can play different roles in plant defense against diverse pathogens. AtGRP7 enhances resistance against a necrotrophic bacterium *Pectobacterium carotovorum* SCC1 or a biotrophic virus *tobacco mosaic virus*. By contrast, AtGRP7 plays a negative role in defense against a necrotrophic fungus *Botrytis cinerea*. These results provide evidence that AtGRP7 is a potent regulator in plant defense response to diverse pathogens, and suggest that the regulation of RNA metabolism by RNA-binding proteins is important for plant innate immunity.

1. Introduction

Plant immune responses, including both pathogen-associated molecular patterns (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI), are mediated by activation and repression of a large array of genes during pathogen infection [1]. Studies on the regulation of gene expression in plant response to pathogens have largely focused on the determination of the transcript levels of genes of interest upon pathogen challenge. In recent years, it is increasingly evident that posttranscriptional regulation of gene expression as well as transcriptional regulation is also crucial for the growth, development, and stress and defense response of plants. Posttranscriptional processes, including pre-mRNA splicing, mRNA transport, mRNA stability and translation, are regulated by diverse RNA-binding proteins (RBPs) and microRNAs (miRNAs). During the last several years, a number of reports have demonstrated that miRNAs serve as a crucial regulator for gene expression reprogramming in plant immune responses [2–8].

Contrary to the increasing understanding on the roles of miRNAs in plant immune responses, reports demonstrating the roles and mechanisms of action of RBPs in gene expression reprogramming during plant immune responses are quite limited. Although it has been determined that the expression of *RBPs* is regulated by wounding, pathogen, salicylic acid (SA), jasmonic acid (JA) or ethylene [9–12], their molecular roles in plant defense against pathogens are just beginning to be uncovered. It has been demonstrated that the RNA-binding protein-defense related 1 (AtRBP-DR1) in *Arabidopsis thaliana* is a positive regulator of SA-mediated immunity, possibly acting on SA signaling-related genes at posttranscriptional level [13]. Two putative RBPs in MOS4-associated complex have been determined as a component contributing to snc-mediated autoimmunity [14].

Glycine-rich RNA-binding proteins (GRPs) are RBPs that harbor the RNA-recognition motif (RRM) at the N-terminus and a glycine-rich region at the C-terminus [15]. Plants GRPs have been identified in a variety of plant species [16–21]. It has been determined that the expression of *GRPs* is regulated by a number of external stimuli including environmental stresses, hormones, light and pathogens [22]. *Arabidopsis* genome has eight member of AtGRPs, and the roles and cellular functions of AtGRPs in abiotic stress responses have been determined for AtGRP2, AtGRP4 and AtGRP7 [23–26]. The AtGRP7 is the most interesting protein among the eight GRP family

Abbreviations: AtGRP7, Arabidopsis glycine-rich RNA-binding protein 7; GRP, glycine-rich RNA-binding protein; RRM, RNA-recognition motif; RBP, RNA-binding protein.

* Corresponding author. Tel.: +82 62 530 2181; fax: +82 62 530 2047.
E-mail addresses: hskang@jnu.ac.kr, hskang@chonnam.ac.kr (H. Kang).

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members present in *Arabidopsis* genome in that it is involved in diverse cellular processes including circadian response, ABA response and stress response [25–29]. In addition, a recent report by Fu et al. [30] points to the importance of AtGRP7 in defense response to *Pseudomonas syringae* pv. tomato DC3000 and to the post-transcriptional control of RNA metabolism as a new pathogenic strategy to suppress innate immunity. It has been demonstrated that the type III effector, HopU1, which is secreted by *P. syringae* pv. tomato DC3000, catalyzes the ADP-ribosylation of several *Arabidopsis* RBPs including AtGRP7, and that the ADP ribosyltransferase activity is necessary for HopU1's ability to suppress PTI and ETI. In recent, it has been determined that the ADP-ribosylation of AtGRP7 by HopU1 decreases the AtGRP7's ability to bind RNA molecules [31].

Plant responses to plant-associated bacteria, both pathogenic and mutualistic, are multifactorial in nature and can vary by bacterial species and strain [1,32,33]. Because AtGRP7 confers plant defense against *P. syringae* [30], we wanted to determine whether AtGRP7 can lead defense against diverse spectrum of pathogens. Here, we show that AtGRP7 is involved in defense response, in addition to *P. syringae*, to other pathogens including a necrotrophic bacterium (*Pectobacterium carotovorum* SCC1; formerly *Erwinia carotovora* SCC1), a necrotrophic fungus (*Botrytis cinerea*) and a biotrophic virus (*Tobacco mosaic virus*, TMV). In contrast to its role in enhancing resistance against *P. syringae*, *P. carotovorum* and TMV, AtGRP7 plays a negative role in disease resistance to *B. cinerea*.

2. Results

2.1. AtGRP7 confers defense against P. carotovorum SCC1 and TMV

Since it has been shown that AtGRP7 confers plant defense against *P. syringae* DC3000 [30], we aimed to determine whether AtGRP7 plays a defensive role against other pathogens. A bacterium *P. carotovorum* and a fungus *B. cinerea* were selected for study because they are necrotrophic pathogens producing cell wall degrading enzymes or toxins as main virulence factors and causing severe disease in numerous plant species [34,35]. We first examined whether the expression of *AtGRP7* is modulated by *P. carotovorum* or *B. cinerea* infection. As shown in Fig. 1, the transcript level of *AtGRP7* was markedly increased in *Arabidopsis* 48 h after *P. carotovorum* infection, whereas its expression was not significantly altered in *Arabidopsis* upon *B. cinerea* challenge. No significant changes in the transcript level of *Actin* were observed, and the expression of a pathogen-responsive marker gene *PDF1.2* was markedly increased in *Arabidopsis* by *P. carotovorum* or *B. cinerea* infection, indicating that the pathogens are adequately applied to the plants.

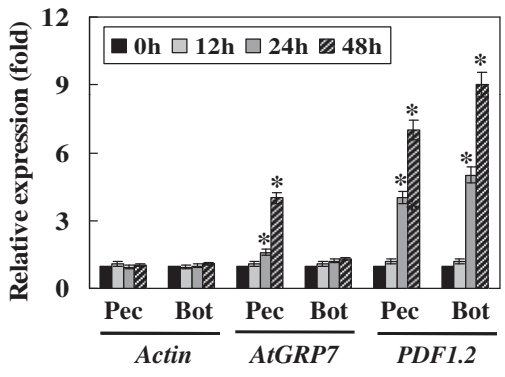

Fig. 1. Expression patterns of *AtGRP7*, *PR-1*, and *PDF1.2* in *Arabidopsis* upon pathogen challenge. Total RNAs were extracted from 3-week-old *Arabidopsis* plants subjected to either *P. carotovorum* SCC1 (Pec) or *B. cinerea* (Bot) infection, and the transcript levels of *AtGRP7*, *PR-1*, and *PDF1.2* were determined via real-time RT-PCR analysis and presented as the relative expression (fold) of the 0 h controls. The values are means ± SE obtained from three independent experiments. Asterisks above the columns indicate values that are statistically different from control values ($P \leq 0.05$).

To determine whether AtGRP7 plays a defensive role against different pathogens, we analyzed the disease resistance of AtGRP7-overexpresing transgenic *Arabidopsis* plants and T-DNA tagged mutant plants. The AtGRP7-overexpresing transgenic *Arabidopsis* plants that constitutively overexpress AtGRP7 under control of the cauliflower mosaic virus 35S promoter and the loss-of-function T-DNA tagged mutant line analyzed in this study have been described in our previous study [26]. Overexpression of AtGRP7 protein in the transgenic plants and the absence of AtGRP7 protein in the knockout mutant were verified by Western blot analysis (Fig. S1). To determine whether AtGRP7 plays a role in the defense response against the soft-rot pathogen *P. carotovorum*, 4-week-old wild-type, 35S::AtGRP7, and *grp7* mutant plants were infected with *P. carotovorum*, and disease severity and the number of bacteria in the leaves were evaluated at 3 days after inoculation (DAI) (Fig. 2). It was evident that the growth of *P. carotovorum* in *grp7* mutant plants was much higher than that in the wild-type plants, whereas the growth of *P. carotovorum* in 35S::AtGRP7 plants was lower than that in the wild-type plants. These results indicate that AtGRP7 plays a positive role in defense against *P. carotovorum*.

Transgenic tobacco plants in which AtGRP7 is expressed under control of the cauliflower mosaic virus 35S promoter were also analyzed. Expression of AtGRP7 in the transgenic tobacco plants was confirmed by RT-PCR and Western blot analyses (Fig. S2). To determine the involvement of AtGRP7 in defense response against TMV, we investigated the AtGRP7-overexpressing transgenic lines (C1 and D1) and the wild-type tobacco plants. After inoculation of TMV, the C1 and D1 transgenic tobacco plants showed a delay in necrotic lesion formation compared with the wild-type plants (Fig. 3). Consequently, the size of necrotic lesions in transgenic lines was much smaller than that in the wild-type plants. The total number of necrotic lesions was also fewer in C1 and D1 transgenic lines compared to the wild-type plants (Fig. 3). These results show that AtGRP7 confers defense against *P. carotovorum* and TMV in *Arabidopsis* and tobacco plants, respectively.

2.2. AtGRP7 enhances the susceptibility of Arabidopsis plant to B. cinerea

We next determined whether AtGRP7 has a role in defense against the necrotrophic pathogen *B. cinerea*. Four-week-old wild-type, 35S::AtGRP7, and *grp7* mutant plants were infected with *B. cinerea*, and disease severity and the density of fungi in the leaves were evaluated at 3 DAI (Fig. 4). The average area of necrotic lesions in the leaves of 35S::AtGRP7 transgenic plants was larger than that in the leaves of the wild-type plants, whereas the average area of necrotic lesions in the leaves of *grp7* mutant plants was smaller than that in the leaves of the wild-type plants. The density of *B. cinerea* in each leaf was quantitatively determined by measuring the amount of *B. cinerea tubulin* by RT-PCR analysis. The RT-PCR results also showed that the density of *B. cinerea* was much higher in 35S::AtGRP7 plants than that in the wild-type plants, whereas the density of *B. cinerea* was slightly lower in *grp7* mutant plants than that in the wild-type plants. These results show that AtGRP7 plays a negative role in defense responses to *B. cinerea* in *Arabidopsis* plants.

2.3. Expression of pathogen-responsive MAPK genes upon pathogen infection

With the observation that AtGRP7 displays a positive and negative roles in defense response against *P. carotovorum* and