Saudi Journal of Biological Sciences 24 (2017) 1884–1893

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Overexpression of *BAS1* in rice blast fungus can promote blast fungus growth, sporulation and virulence in *planta*

Jing Yang, Lin Liu, Yunfen Wang, Chunmei Wang, Jinlu Yan, Yanqin Liu, Changmi Wang, Chengyun Li *

State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming, Yunnan, China

ARTICLE INFO

Article history:
Received 30 September 2017
Revised 9 November 2017
Accepted 10 November 2017
Available online 11 November 2017

Keywords:
Magnaporthe oryzae
Rice
Effector proteins
Defense-related genes
BIC

ABSTRACT

Background: BAS1 is biotrophy-associated secreted protein of rice blast strain (*Magnaporthe oryzae*). In order to study the effect of BAS1 on virulence of rice blast strain, we characterized function of BAS1 using a purified prokaryotic expression product of *BAS1* and its overexpression strain. *Results:* Our results showed *in vitro* the purified expression product caused rapid callose deposition and ROS production in rice leaves and calli, indicated it triggered transient basal defense. When the purified expression product of BAS1 was sprayed onto rice leaves, and 24 h later the leaves were inoculated with blast strain, the results showed the size and number of lesions, on purified BAS1 product-pretreated leaves of the Lijiangxintuanheigu (LTH) challenged with blast strain, was higher than those in BAS1-untreated leaves directly challenged with the same strain, which suggested the defense response trigged by BAS1 can be overcome by other effectors of the fungus. More severe symptoms, higher sporulation, higher relative fungal growth and more lower expression level of defense-related genes appeared in LTH leaves challenged with overexpression strain 35S:BAS1/Mo-2 than those in LTH inoculated with wild-type strain. *Conclusions:* These data suggest both *in vitro* pretreatment with *BAS1* prokaryotic expression products and overexpression in blast strains can increase virulence of blast fungus.

© 2017 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

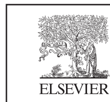
1. Introduction

The immune response capabilities of plants have evolved to respond to and resist pathogen infection. Host plants recognize pathogen associated molecular patterns (PAMPs) such as flagellin, lipopolysaccharide, glycoprotein, and chitin as the first line of the defense response (Dangl and Jones, 2001). These receptors encode transmembrane receptor-like kinases. Once the receptor recognizes the PAMP, it triggers a series of immune responses in the host plant. These responses include stomatal closure, MAPK activation, ROS generation, differential gene expression, callose deposition, and other physiological processes, which lead to a basic immune response or PAMP-triggered immunity (PTI) (Melotto et al., 2006; Navarro et al.,2004; Schwessinger and Zipfel, 2008; Zipfel et al., 2004). In contrast, the pathogen secretes a large number of effector proteins that inhibit PTI to invade and colonize the host (Göhre and Robatzek, 2008; Lindeberg et al., 2009).

Pathogenic bacteria infect plants, and produce type III effector proteins in order to suppress the immune response (Büttner and He, 2009). Bacterial type III secretary effector (T3SE) has multiple functions, which can transport into the host cell and reprogram multiple metabolic pathways, such as the induction of defense-related gene expression, downstream defense signal activation, specific protein modification, as well as production of SA, JA, and Et signaling molecules, etc. (Stulemeijer and Joosten, 2008). Overexpression of the type III effector protein in plants can alter the plant response to the pathogen, either resulting in plant infection or inducing the plant defense response. AvtaBsT, identified from bacterial scab disease, is the first type III effector protein of the YopJ family. Effector proteins in the YopJ family mainly inhibit host ETI response (Büttner and He, 2009) while AvrBsT is required for HR induction of resistance in response to pathogen infection (Büttner and He, 2009). When bacterial scab pathogens infect host plants, AvrBsT is transported into plant cells to initiate defense responses (Kirik and Mudgett, 2009). GST (Glutathione S transferase)-AvrBsT fusion protein induces leaf cell death in *Arabidopsis* seedlings (Hwang et al., 2012), and its transient expression causes hypersensitive cell death in tobacco and pepper leaves (Kim et al., 2010), However, certain concentrations of

* Corresponding author.
E-mail address: lichengyun@gmail.com (C. Li).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2017.11.032
1319-562X/© 2017 Production and hosting by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

GST-AvrBsT fusion protein apparently decrease fungi infection in young leaves of Arabidopsis (Hwang et al., 2012). The effector protein AvrBsT mitigates pathogen infections *in vitro*, but the underlying mechanism for the induction of the basal defense response(s) remains unclear.

The pathogenicity of *Magnaporthe oryzae* to rice, is well studied, yet rice blast disease still threatens global rice production and food security in many rice growing regions (Wang and Valent, 2009). *M. oryzae* secretes large amounts of effector proteins during the infection of rice tissue, which facilitates the penetration of the fungus into the host epidermal cells, evading host recognition, and reprogramming host defense genes, to create a favorable environment for the growth and reproduction of the pathogen. The interaction between this hemibiotrophic fungus and its host is a complex system involving infection hyphae and the host membrane system termed the BIC (biotrophic interface complex). Both the host and the pathogen regulate the structure and function of this complex system. The fungi secrete a large amount of the effector proteins, including PWL2 and Avr-Pita in the biotrophic phase. A large number of genes were up-regulated in the biotrophic phase of fungal growth have been designated as biotrophy-associated secreted (BAS) proteins (Mosquera et al., 2009). BAS1-4 have been widely studied, but their function within the fungus and the host tissues is still not clear, and whether the function of BAS effectors are limited to the biotrophic stage of infection is of particular interest. The expression of BAS1 is upregulated 100-fold in the fungal infection hyphae (IH) (Mosquera et al., 2009), which can enter the rice cytoplasm (Ribot et al., 2013; Gao et al., 2017). In the present study, we investigated the effect of a BAS1-overexpression strain and the prokaryotic expression product of GST-BAS1-mCherry on the susceptible rice variety LHT. We also tested the effect of BAS1 on the infection of *M. oryzae* strains *in vitro* by pretreating rice leaves for 24 h using GST-BAS1-mCherry before inoculating the leaves with a conidial suspension. Moreover, we tested the pathogenicity, hyphal growth and sporulation *in planta* of the BAS1 overexpressed transformants and the expression levels of defense-related genes in rice leaves challenged with the BAS1 overexpression strain. The objective of which was to determine the role of BAS1 in the blast fungus infection process.

2. Materials and methods

2.1. Rice blast strain and rice cultivar

We used the rice variety, Lijiangxintuanheigu (LTH) that is highly susceptible to *M. oryzae* strains. The *M. oryzae* strains used in this study was 66b (strong pathogenic strain), BAS1 overexpressing strain under 35S promoter (35S:BAS1/Mo-2) (the overexpression strain was got previously), and wild-type strain 95234I-2b (PCR analysis showed that this strain did not harbor the *BAS1* gene). All these strains were preserved in our laboratory. GST-BAS1-mCherry was the prokaryotic expression product used for spraying rice seedlings.

2.2. Activation of M. oryzae and preparation of spore suspension

Mycelia of *M. oryzae* were inoculated on petri plates containing PDA solid medium (potato 200 g, glucose 20 g, agar 15 g, and water 1000 ml), which was cultured in a 28 °C incubator until the mycelia covered the entire agar surface. Mycelium blocks were transferred to a flask, which was cultured in a 28 °C shaker for 5–7 d, and then stored in 4 °C refrigerator prior to use. The mycelium liquid of *M. oryzae* was spread out evenly on petri plates containing tomato-oat medium (tomato-oat medium: tomato juice 300–400 ml, oats 40 g, $CaCO_3$ 0.6 g, agar 20 g, and water 1000 ml). The plates were incubated at 25 °C for 7–10 days to sporulate. Approximately, 20 ml of sterile water was added into the dish, and then the plates were gently scraped, washed, and filtered to obtain the spore suspension. The concentration was adjusted to 1×10^5 cells/ml.

2.3. Cultivation of rice seedlings and leaf inoculation

Rice seeds were sterilized with 1.5% sodium hypochlorite and incubated at 28 °C for germination. The germinated seedlings were sown in a seedling tray. When the rice grew to the 3–4 leaf stage, it was moved to an inoculation box. Spore suspension of *M. oryzae* was sprayed on the rice and sufficient moisture was provided for 24 h after which the seedlings were transferred to a greenhouse. Disease incidence was investigated at six days, and leaf samples were collected at different times. Three repeats were performed for each treatment, and 15 seedlings were surveyed for each repeat. Four seedlings were sampled for each repeat at each time point.

2.4. Real-time RT-PCR analysis of defense-related genes in rice leaves

Total RNA was extracted using the TRIZOL (Invitrogen) extraction kit. Total RNA was reverse transcribed using Superscript III (Invitrogen) to obtain cDNA. Real-time RT-PCR primer sequences for the defense-related genes in rice a shown in Table 1.

Real-time PCR (Bio-Rad) 25.0 µl reaction system: 2.0 µl template cDNA, 0.5 µl forward primer, 0.5 µl reverse primer, 12.50 µl 2 × EasyTaq PCR SuperMix, and 9.5 µl sterilized ddH_2O. Amplification cycle parameters: pre-denaturation at 95 °C for 3 min, denaturation at 95 °C for 20 s, annealing extension at 59 °C for 20 s, and a collection of fluorescence signals at 65 °C; a total of 44 cycles were performed. Dissolution curve parameters: the temperature was increased starting from 59 °C; fluorescent signals were collected at each cycle with the temperature increased by 0.5 °C, and a total of 80 cycles were performed. Three repeats were performed for each sample. Ct values were recorded to calculate the relative expression levels. Real-time PCR data was analyzed with the $2^{-\triangle\triangle Ct}$ method. Expression levels of the resistance genes in rice were calculated. The relative gene expression level = treated sample (target gene Ct – actin Ct) – blank sample (target gene Ct – actin Ct).

2.5. Callose and ROS observation

LTH blades were selected at the two-leaf stage, and shortened to 4 cm length. The blades were then immersed in clear water for 2 h and then placed on wet filter paper in a petri dish. The

Table 1
Disease incidence on leaves inoculated with blast strain.

Treatment	Disease incidence (%)
BAS1/66b	39.81 ± 2.49
PBS/66b	28.24 ± 2.33
66b	25.67 ± 1.68
BAS1/Guy11	26.19 ± 1.52
PBS/Guy11	20.28 ± 2.15
Guy11	17.05 ± 1.01

Note: BAS1/66b indicated leaves that treated with BAS1 solution were challenged with blast strain of 66b; PBS/66b as control indicated leaves that treated with PBS solution were challenged with 66b; 66b as control indicated leaves were challenged with 66b. BAS1/Guy11 mean leaves that treated with BAS1 solution were challenged with blast strain of Guy11; PBS/Guy11 as control indicated leaves that treated with PBS solution were challenged with Guy11; Guy11 as control indicated leaves were challenged with Guy11.

Download English Version:

https://daneshyari.com/en/article/8850060

Download Persian Version:

https://daneshyari.com/article/8850060

Daneshyari.com