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Induced defense responses in rice plants against small brown planthopper infestation

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ABSTRACT

The small brown planthopper (SBPH), *Laodelphax striatellus* Fallén (Homoptera: Delphacidae), is a serious pest of rice (*Oryza sativa* L.) in China. To understand the mechanisms of rice resistance to SBPH, defense response genes and related defense enzymes were examined in resistant and susceptible rice varieties in response to SBPH infestation. The salicylic acid (SA) synthesis-related genes phenylalanine ammonia-lyase (*PAL*), *NPR1*, *EDS1* and *PAD4* were induced rapidly and to a much higher level in the resistant variety Kasalath than in the susceptible cultivar Wuyujing 3 in response to SBPH infestation. The expression level of *PAL* in the Kasalath rice at 12 h post-infestation (hpi) increased 7.52-fold compared with the un-infested control, and the expression level in Kasalath was 49.63, 87.18, 57.36 and 75.06 times greater than that in Wuyujing 3 at 24, 36, 48 and 72 hpi, respectively. However, the transcriptional levels of the jasmonic acid (JA) synthesis-related genes *LOX* and *AOS2* in resistant Kasalath were significantly lower than in susceptible Wuyujing 3 at 24, 36, 48 and 72 hpi. The activities of the defense enzymes PAL, peroxidase (POD), and polyphenol oxidase (PPO) increased remarkably in Kasalath in response to SBPH infestation, and were closely correlated with the *PAL* gene transcript level. Our results indicated that the SA signaling pathway was activated in the resistant Kasalath rice variety in response to SBPH infestation and that the gene *PAL* played a considerable role in the resistance to SBPH.

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Abbreviations: AOS2, allene oxide synthase 2; *EDS1*, enhanced disease susceptibility 1; *EIN2*, ethylene insensitive 2; ET, ethylene; hpi, hours post-infestation; JA, jasmonic acid; *LOX*, Lipoxygenase; *NPR1*, nonexpressor of pathogenesis-related genes 1; *PAD4*, phytoalexin deficient 4; PAL, phenylalanine ammonia-lyase; POD, peroxidase; PPO, polyphenol oxidase; *PR1b*, pathogenesis related protein 1b; SA, salicylic acid; SAR, systemic acquired resistance; SBPH, small brown planthopper.

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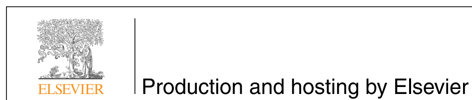

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1. Introduction

The small brown planthopper (SBPH), *Laodelphax striatellus* Fallén, is a serious sap-sucking pest of rice (*Oryza sativa* L.) in China and other parts of East Asia extending to Indonesia, the Philippines and Vietnam. Leaves infested by SBPH turn yellow, become wilted, and even die, resulting in yield loss and quality reduction. Furthermore, the SBPH also transmits rice viral diseases such as *Rice stripe virus* (RSV) and *Rice black-streaked dwarf virus* (RBSDV), which often cause major additional yield losses apart from just the damage by the insect itself [1–3]. Currently, pesticides are widely used to control the SBPH, but this leads to the death of natural enemies, environmental pollution, chemical resistance and resurgence [4]. Therefore, host-plant resistance has been recognized as one of the most economic, effective and environmentally-friendly measures for controlling SBPH [5,6].

Plant responses to herbivores are regulated through a complex network of signaling pathways that involve three signaling molecules: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) [7,8]. Generally, the JA pathway is considered to be required for defense against necrotrophic pathogens and chewing insects, while the SA pathway is involved in a wide range of plant defense responses [9–11]. Herbivore feeding behaviors primarily involve chewing and sucking. The beet armyworm (*Spodoptera exigua* Hübner) is a typical chewing pest, whose herbivory can cause large scale leaf damage. Some elicitors such as volicitin from beet armyworm oral secretions can provoke defense reactions to wounding mediated by the JA signaling pathway [12,13]. Sucking insects such as phloem-feeding whiteflies and aphids that cause little injury to plant foliage are perceived as pathogens and primarily activate SA-dependent and to a certain extent JA/ET-dependent signaling pathways [7,14,15].

Plant defense is usually induced when subjected to pathogens, insects or wounding. Induced resistance can be split broadly into systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR develops systemically in response to, for example, pathogen infection or treatment with certain chemicals (e.g., 2,6-dichloroisonicotinic acid). This acquired resistance is effective against a wide range of pathogens and is mediated by a SA-dependent process [16]. For SAR, many plant enzymes are involved in defense reactions against biotic stresses. Phenylalanine ammonia-lyase (PAL) is the first enzyme of the phenylpropanoid pathway and is involved in the biosynthesis of phenolics, phytoalexins, and lignins, which increase plant resistance [17,18]. Oxidative enzymes such as peroxidase (POD) and polyphenol oxidase (PPO) catalyze the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure [19]. Therefore, defense enzymes such as PAL, PPO and POD are tightly correlated with resistance to pests [20].

Currently, information about rice defense response mechanisms to SBPH, a typical phloem sap-sucking pest, is very limited. Therefore, elucidating the interaction between rice and SBPH would be helpful to understand the molecular basis for plant resistance to sap-sucking insects. In this paper, real-time PCR was used to analyze differential expression of genes involved in the SA- and JA/ET-mediated defense pathways at different time points when resistant and susceptible rice plants were infested by SBPH. Defense enzyme activities were also assayed after SBPH feeding.

2. Materials and methods

2.1. Rice varieties and insect infestation

An *indica* rice variety, Kasalath, and a *japonica* cultivar, Wuyujing 3, were selected for their high resistance and susceptibility to SBPH with the resistance scales of 2.0 and 9.0, respectively [21]. Seeds for these varieties were provided by the Institute of Crop Science at the Chinese Academy of Agricultural Sciences.

The SBPH population used for infestation was originally collected from a rice field in Nanjing, China, and had been maintained on barley in a greenhouse for four generations before being transferred to Wuyujing 3 rice in the greenhouse of the Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China. The SBPH population was confirmed to be non-viruliferous by dot-immunobinding assay and PCR detection [21].

Twenty-five germinated seeds were sown in a plastic pot of 10 cm-diameter and 9 cm-height with a hole in the base. A total of 24 pots were randomly placed in a 65 cm × 44 cm × 14 cm plastic seed-box. All seeds and seedlings for testing were incubated at 26 ± 1 °C with sunlight and natural ventilation. About 2-cm of water level was maintained in the seed-box.

At the 3-leaf stage, the seedlings were infested with second to third instar SBPH nymphs that were starved for 2 h prior to infestation. The rate of infestation was 20 insects per seedling. Rice leaves were collected for RNA extraction at 12, 24, 36, 48 or 72 h post infestation (hpi). Leaves without SBPH infestation were used as a control.

2.2. Isolation of total RNA and first-strand cDNA synthesis

Total RNA was extracted with RNAprep Plant kits (Tiangen Corporation, China), and then treated with RQ1 RNase-Free DNase (Promega, USA) before reverse transcription (RT). First-strand cDNA was synthesized using M-MLV Reverse Transcriptase kits (Promega).

2.3. Real-time quantitative PCR

Real-time quantitative PCR was performed using an ABI PRISM 7300 cycler (Bio-Rad Corporation, USA) with a SYBR Premix (SYBR Green) PCR kit (Tiangen). The primer pairs listed in Table 1 were used to amplify the corresponding 11 genes of interest. Amplification reactions were carried out in a 20 μL volume mixture containing 10 μL of 2 × SuperReal Premix, 0.2 $\mu mol\ L^{-1}$ of each primer, 20 ng of DNA template, 2 μL of 50 × ROX Reference Dye and 6.2 μL of RNase-Free ddH_2O. Template denaturation was conducted for 15 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s and extension at 72 °C for 40 s. Each sample was

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