Isolation and Characterization of Rice OsHRL Gene Related to Bacterial Blight Resistance

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The expression of HR-like lesion inducing gene of *Oryza sativa* (OsHRL) was slightly increased by *Xanthomonas oryzae* pv. *oryzae* (Xoo) infection. Transgenic rice plants over-expressing OsHRL gene were challenged with Xoo and the development of disease symptoms were examined to investigate the effect of OsHRL gene expression on plant defense responses. The over-expression of OsHRL increased disease resistance against Xoo compared with wild type plants.

**Keywords**: avrBs2 homologue, OsHRL, rice, *Xanthomonas oryzae* pv. *oryzae*

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Rice is one of the most important crop species, and its yield is constantly affected by several major diseases such as bacterial blight, blast, sheath blight, and tungro (Dai et al., 2007). Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a serious agronomic problem in many rice-growing regions (Mew et al., 1993). Therefore, understanding of molecular mechanisms underlying host resistance to pathogens is essential to develop better controlling strategies against rice diseases. Because of availability of the genome sequences, rice (Sasaki et al., 2005) and *Xoo* (Lee et al., 2005) are considered as a model system of plant and bacterial pathogen, respectively (Li et al., 2006).

It has been previous reported that the avrBs2 gene, which was identified in many species of *Xanthomonas*, encodes agrocinopine synthase and glycerol phosphodiesterase (Swords et al. 1996). The avrBs2 of *Xanthomonas campestris* pv. *vesicatoria* (Xcv) is specifically recognized by pepper Bs2 resulting in localized cell death and plant resistance (Mudgett et al., 2000). We thus attempt to identify avrBs2-interacting partner from rice. AvrBs2 (Kearney and Staskawicz, 1990; Swords et al., 1996) homologue was selected from *Xoo* strain KACC10859 (K1 race), based on genomic sequences of *Xoo* strain KACC10331 (K1 race, Lee et al., 2005). *Xoo* strain KACC10859 harbors single copy gene of avrBs2 which is used as bait to isolate interacting host components using yeast two-hybrid screening. Possible involvement in plant immunity of one selected gene, OsHRL encoding HR-like lesion inducing protein, was evaluated.

Rice (*O. sativa* L. cv. Dongjin) seedlings were grown for 6 weeks in a greenhouse under controlled temperature (20-30°C) and natural light conditions. For bacterial inoculations, *Xoo* strain was grown in Peptone Sucrose medium at 28°C (Karganilla et al., 1973). The cells were collected by centrifugation, and diluted to OD\(_{600}\)=1.0 in 10 mM MgCl\(_2\). The clipping method was used for inoculation (Kauffman et al., 1973; Mundt et al., 2002; Wu et al., 2007). Leaf and leaf sheath (shoot) were harvested, and used immediately or stored at −80°C for total RNA extraction. Harvested samples were ground to fine powder in liquid nitrogen and total RNA was extracted using RNasy Plant Mini Kit (Qiagen, Hilden) according to the manufacturer’s instructions.

The prey library was constructed with rice cv. Dongjin infected with *Xoo* strain KACC10331 three hours after inoculation. From thirty positive clones, thirteen genes interacting with avrBs2 homologue were identified and OsHRL (*Oryza sativa* HR-like lesion inducing) gene was chosen for further characterization (Fig. 1).

The OsHRL gene contains 474 bp encoding 157 aa with a predicted molecular mass of 17 kDa (Accession number; FJ548850), containing signal peptide (21 aa) and conserved HR-lesion domain (Fig. 2A). A blast search (BLASTP) on the GenBank database using the predicted amino acids sequence as query showed homology to several HR like lesion-inducing proteins of other plants. We compared the OsHRL with other evolutionary related proteins from the reference plants tobacco, pepper, alfalfa and Arabidopsis. We found that rice OsHRL is closely related to *Nicotiana tabacum* HR like lesion-inducing protein (AAC49975), and *Arabidopsis thaliana* elicitor like protein (CAB10221) (Fig. 2B).
The expression level of OsHRL was analyzed after inoculation with *Xoo* strain KACC10859. Leaf and leaf sheath were harvested at 1, 3, 6, 12, 24, and 48 hours after inoculation, from which total RNA was extracted as described above. For RT-PCR, 1 µg of total RNA were reverse transcribed with M-MLV reverse transcriptase (Invitrogen, USA). The PCR program was consisted of 25 cycles using specific primers 5'-ATGGGGTTCGTCTCCTTCG-3' and 5'-CTAGTTCGTCTTCGACTTGGGA-3' for OsHRL and 5'-TGCTATGTACGTCGCCATCCAG-3' and 5'-AATGAGTGTAACCACGCTCCGTCA-3' for *OsActin* as a control.

OsHRL was slightly increased 1 hr after treated with *Xoo* strain KACC10859. *OsActin* was used internal control.
and clipping method. (B) Average lesion lengths produced on ‘Dongjin’ with wild type rice cv. Dongjin is susceptible to Xoo strain KACC10859 or Bs2 in rice cv. Dongjin does not function properly. Recent report provided us with clues for this enigma (Büttner & Bonas, 2010) that AvrBs2 in Xanthomonas campestris pv. vesicatoria (Xcv) did not show phosphodiesterase activity but had virulence function. They all together, the recognition of avrBs2 directly or indirectly by OsHRL makes rice more resistant against Xoo. However, the mechanism explaining the role of OsHRL in disease-resistance needs to be studied further.

In conclusion, we have isolated OsHRL gene from rice via yeast two-hybrid system using avrBs2 homologue from Xoo strain KACC10859 as bait. By RT-PCR analysis, the transcription level of OsHRL was increased by Xoo infection. Transgenic rice plants over-expressing OsHRL displayed increased resistant against Xoo compared with wild type rice.

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References


