Notes

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

Sang Ryeol Park^{1*}, Seok Jun Moon¹, Dongjin Shin¹, Min Gab Kim¹, Duk-Ju Hwang¹, Shin-Chul Bae¹, Jeong-Gu Kim², Bu Young Yi³ and Myung Ok Byun¹

¹Bio-crop Development Division and ²Genomics Division, National Academy of Agricultural Science, RDA, Suwon 441-707, Korea

³Department of Environmental Horticulture, University of Seoul, Seoul 130-743, Korea

(Received on June 21, 2010; Accepted on November 2, 2010)

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*

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 previously 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 OD₆₀₀=1.0 in 10 mM MgCl₂. 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 RNeasy 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).

*Corresponding author.

Phone) +82-31-299-1743, FAX) +82-31-299-1722
E-mail) srpark@korea.kr

Fig. 1. Confirmation of *avrBs2* and *OsHRL* interaction by yeast two-hybrid screening. (A) On SD/-His/-Leu/-Trp media. (B) On SD/-Ade/-His/-Leu/-Trp/X-α-Gal media. +; pGADT7-T/pGBKT7-53 was a positive control, -; pGADT7-T/pGBKT7-Lam was a negative control, positive clones were pGBKT7-*avrBs2*/pGADT7-*OsHRL*.

Fig. 2. Nucleotide sequence and phylogenetic relationship of amino acids sequence of *OsHRL*. (A) Nucleotide sequence and deduced amino acids sequence of *OsHRL* gene. The signal peptide was shaded and the HR-lesion domain was boxed. (B) The phylogenetic tree of *OsHRL* and other related proteins. One thousand bootstrap replicates were performed. The rice *OsHRL* sequence was aligned with *Nicotiana tabacum* HR like lesion-inducing protein (*NtHRL*, AAC49975), *Arabidopsis thaliana* elicitor like protein (*AtELP*, CAB10221), *Medicago sativa* HR lesion-inducing ORF (*MsHRL*, AAC77929) and *Capsicum annuum* putative lesion inducing protein (*CaLIP*, AAR83861) using DNAMAN CLUSTAL-W programs.

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

Fig. 3. Expression analysis of *OsHRL* in mock (10 mM $MgCl_2$) and *Xoo* strain KACC10859 treated rice cv. Dongjin. Six-week-old rice seedlings were infected with *Xoo* strain KACC10859, and then leaves were harvested at 0, 1, 3, 6, 12, 24 and 48 hr. Total RNA was isolated and RT-PCR was performed using *OsHRL* specific primer pairs. *OsHRL* was slightly increased from 1h after treated with *Xoo* strain KACC10859. *OsActin* was used internal control.

inoculation, from which total RNA was extracted as described above. For RT-PCR, 1 μ g 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'-AATGA-GTAACCACGCTCCGTC-3' for *OsActin* as a control. The transcription level of *OsHRL* was slightly increased 1 hr after inoculation with *Xoo* strain KACC10859 (Fig. 3).

To analyze its possible involvement in disease resistance, we generated transgenic rice plants by *Agrobacterium* mediated transformation method (Hiei et al., 1994), in which *OsHRL* was over-expressed under controlled of CaMV 35S promoter in pB7WG2D Gateway vector (Invitrogen). Transgenic rice plants cv. Dongjin were transplanted from medium to soil, and screened by sparying the plants with solution of 0.1 mg/ml Basta 14 days after transplanting (Jiang et al., 2000; Ramesh et al., 2004). The seeds were collected and planted to obtain next generation transgenic plants. T2 transgenic plants were used in further study.

The 6 week-old T2 transgenic rice seedlings were inoculated with *Xoo* strain KACC10859, and the lesion length was monitored 14 days post-inoculation (dpi). The five leaves of each line were monitored and three independent

Fig. 4. Evaluation of disease resistance to *Xoo* strain KACC10859 of the *OsHRL*-overexpressing rice and the wild type rice. (A) Representative leaves showing lesions produced on wild type rice cv. 'Dongjin' and the *OsHRL*-overexpressing T2 rice plants 14 days post-inoculation with *Xoo* strain KACC10859 by the leaf clipping method. (B) Average lesion lengths produced on 'Dongjin' and *OsHRL*-overexpressing T2 rice plants 14 days post-inoculation with *Xoo* strain KACC10859. *OsHRL*-overexpressing T2 transgenic rice plants were more resistant than wild type. (C) RT-PCR analysis of wild type rice cultivar 'Dongjin' and *OsHRL*-overexpressing rice plants (T2). *OsHRL*-overexpressing rice plants (T2) showed high level of *OsHRL* expression compared with wild type.

experiments exhibited similar results. The six-transgenic plants (2-2-9, 2-3-2, 2-4-1, 2-6-1, 5-3-1, 5-4-1) were more resistant than wild type plants after infection with *Xoo* strain KACC10859 (Fig. 4A & B), and they showed high level expression of *OsHRL* compared to wild type (Fig. 4C).

Plants are continuously attacked by various phytopathogenic bacteria and thus evolved an innate immune system to cope with those invaders. Two mode of plant immunity against pathogen, effector-triggered immunity (ETI) and pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), are induced by recognition of pathogen effectors and PAMPs, respectively (Jones and Dangl, 2006).

We have shown that high level expression of *OsHRL* conferred rice on bacterial leaf blight disease resistance. Based on this, we have postulated that the resistance against *Xoo* comes from the recognition of AvrBs2 mediated by high level of *OsHRL*, suggesting ETI might play pivotal role rather than PTI. However, rice cv. Dongjin is susceptible to *Xoo* KACC10859 which is expressing AvrBs2, even though this rice cultivar contains Bs2 like-resistance gene. It is not in consonance with the basic concept that the recognition of effector protein itself or the activity of effector mediated by host component confers resistance on plant. It can be thus explained by two different ways, either

AvrBs2 in *Xoo* 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.

Acknowledgement

This work was supported by a grant from the National Academy of Agricultural Science (200901FHT020812415), Rural Development Administration, and grant (CG3134-1) from the Crop Functional Genomics section of the 21st Century Frontier Research Program, Ministry of Education, Science and Technology Republic of Korea.

References

- Büttner, D. and Bonas, U. 2010. Regulation and secretion of *Xanthomonas* virulence factors. *FEMS Microbiol. Rev.* 34:107-133.
- Dai, L. Y., Liu, X. L., Xiao, Y. H. and Wang, G. L. 2007. Recent advances in cloning and characterization of disease resistance genes in rice. *J. Integr. Plant Biol.* 49:112-119.
- Hiei, Y., Ohta, S., Komari, T. and Kumashiro, T. 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant J.* 6:271-282.
- Jiang, J., Linscombe, S. D., Wang, J. and Oard, J. H. 2000. High efficiency transformation of U.S. rice lines from mature seed-derived calli and segregation of glufosinate resistance under field conditions. *Crop Sci.* 40:729-1741.
- Jones, J. D. and Dangl, J. L. 2006. The plant immune system. *Nature* 444:323-329.
- Karganilla, A., Paris-Natural, M. and Ou, S. H. 1973. A comparative study of culture media for *Xanthomonas oryzae*. *Philipp. Agric.* 57:141-152.
- Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y. and Merca, S. D. 1973. An improved technique for evaluation of resistance of rice varieties to *Xanthomonas oryzae*. *Plant Dis. Repotr.* 57: 537-541.
- Kearney, B. and Staskawicz, B. J. 1990. Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*. *Nature* 346:385-386.

- Lee, B. M., Park, Y. J., Park, D.-S., Kang, H.-W., Kim, J.-G., Song, E.-S., Park, I.-C., Yoon, U.-H., Hahn, J.-H., Koo, B.-S., Lee, G.-B., Kim, H., Park, H.-S., Yoon, K.-O., Kim, J.-H., Jung, C.-H., Koh, N.-H., Seo, J.-S. and Go, S.-J. 2005. The genome sequence of *Xanthomonas oryzae* pathovar *oryzae* KACC10331, the bacterial blight pathogen of rice. *Nucleic Acids Res.* 33:577-586.
- Li, Z. K., Arif, M., Zhong, D. B., Fu, B. Y., Xu, J. L., Domingo-Rey, J., Ali, J., Vijayakumar, C. H. M., Yu, S. B. and Khush, G. S. 2006. Complex genetic networks underlying the defensive system of rice (*Oryza sativa* L.) to *Xanthomonas oryzae* pv. *oryzae*. *Proc. Natl. Acad. Sci. USA* 103:7994-7999.
- Mudgett, M. B., Chesnokova, O., Dahlbeck, D., Clark, E. T., Rossier, O., Bonas, U. and Staskawicz, B. J. 2000. Molecular signals required for type III secretion and translocation of the *Xanthomonas campestris* AvrBs2 protein to pepper plants. *Proc. Natl. Acad. Sci. USA* 97:13324-13329.
- Mew, T. W., Alvarez, A. M., Leach, J. E. and Swings, J. 1993. Focus on bacterial blight of rice. *Plant Dis.* 77:5-12.
- Mundt, C. C., Nieva, L. P. and Vera Cruz, C. M. 2002. Variation for aggressiveness within and between lineages of *Xanthomonas oryzae* pv. *oryzae*. *Plant Pathol.* 51:163-168.
- Ramesh, S., Nagadhara, D., Reddy, V. D. and Rao, K. V. 2004. Production of transgenic indica rice resistant to yellow stem borer and sap-sucking insects, using super-binary vectors of *Agrobacterium tumefaciens*. *Plant Sci.* 166:1077-1085.
- Sasaki, T., Matsumoto, T., Antonio, B. A. and Nagamura, Y. 2005. From mapping to sequencing, post-sequencing and beyond. *Plant Cell Physiol.* 46:313.
- Swords, K. M., Dahlbeck, D., Kearney, B., Roy, M. and Staskawicz, B. J. 1996. Spontaneous and induced mutations in a single open reading frame alter both virulence and avirulence in *Xanthomonas campestris* pv. *vesicatoria* avrBs2. *J. of Bacteriol.* 178:4661-4669.
- Wu, X. M., Li, Y. R., Zou, L. F. and Chen, G. Y. 2007. Gene-for-gene relationships between rice and diverse avrBs3/*pthA* avirulence genes in *Xanthomonas oryzae* pv. *oryzae*. *Plant Pathol.* 56:26-34.