Intellectual Contribution

Molecular mechanism of *Pb1*-mediated panicle blast resistance in rice

Haruhiko Inoue, Nagao Hayashi, Akane Matsushita, Xinqiong Liu, Shoji Sugano, Chang-Jie Jiang, Hiroshi Takatsuji

Disease Resistant Crops Research Unit

We elucidated the action mechanism of panicle blast resistance due to *Pb1* gene. Degradation of WRKY45, which plays a central role in induced resistance, was suppressed upon binding with the Pb1 protein leading to strong blast resistance. This mechanism accounts for the durability of Pb1-dependent blast resistance.

Keywords: rice, blast disease, blast resistance, WRKY45

Background

In Japan, an average of 22 billion yen is spent annually for agrochemicals in order to control rice damage caused by the blast disease. Thus, blast resistance is one of the most important targets of rice breeding. Several blast resistance genes have been identified so far. However, many of them are effective against particular races of blast fungi only and the resistance against the blast disease is often lost by breakdown within a few years. The *Panicle blast 1* (*Pb1*) gene has been utilized for rice breeding because of its durability. However, the reason for the durability has been unknown. It is therefore necessary to clarify the molecular mechanism involved in *Pb1*-mediated resistance against blast so that it can be used efficiently and safely in the future. In this study, we investigated the relationship of *Pb1* with WRKY45, the central transcription factor involved in induced disease resistance of rice.

Results and Discussion

1. The Pb1 protein has a CC-NBS-LRR structure similar to R-proteins for true blast resistance, and interact with WRKY45, the central transcription factor involved in induced disease resistance of rice (Fig. 1).
2. Analysis of *Pb1*-containing rice in which *WRKY45* was suppressed by RNAi demonstrated that the blast resistance by Pb1 was dependent on WRKY45 (Fig. 2).
3. Addition of a nuclear-exclusion signal to Pb1 protein abolished *Pb1*-dependent blast resistance, indicating that Pb1 protein functions in the nucleus (Fig. 3).
4. We have previously shown that WRKY45 is degraded by proteasome, resulting in the reduction of WRKY45-dependent blast resistance. In this study, we showed that the binding of Pb1 protein to WRKY45 protein suppressed the WRKY45 degradation (Fig. 4A).
5. These results indicate that in *Pb1*-containing rice, the degradation of WRKY45 was suppressed upon binding with Pb1 leading to strong blast resistance (Fig. 4B).
6. This study elucidated the mechanism of *Pb1*-dependent blast resistance that is mediated by suppression of WRKY45 degradation. This mechanism at least partly accounts for the race-non-specificity and durability of *Pb1*-dependent blast resistance.

Future prospects

1. The mechanism of *Pb1*-dependent blast resistance elucidated in this study indicates that it is unlikely to breakdown. This information will contribute to prevalence of *Pb1* in rice breeding.
2. Strengthening the binding ability of Pb1 protein to WRKY45 should lead to the development of a highly stable and durable disease resistant rice.

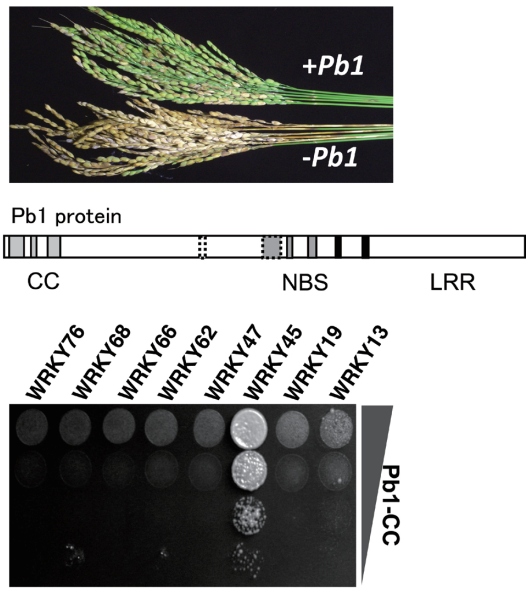

Fig. 1. Analysis of the Pb1 protein structure and binding with WRKY45. The Pb1 protein has a coiled-coil–nucleotide- binding site–leucine-rich repeat (CC-NBS-LRR) structure. Pb1 binds specifically to WRKY45.

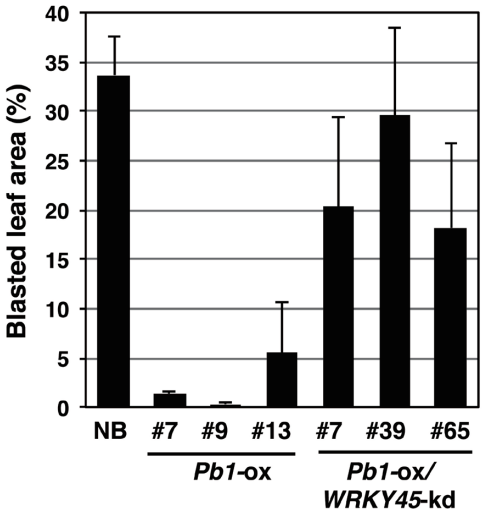

Fig. 2. WRKY45-dependent blast resistance of Pb1. Knockdown of the *WRKY45* gene (*WRKY45*-kd) abolished *Pb1*-dependent blast resistance in *Pb1*-overexpression (*Pb1*-ox) rice.

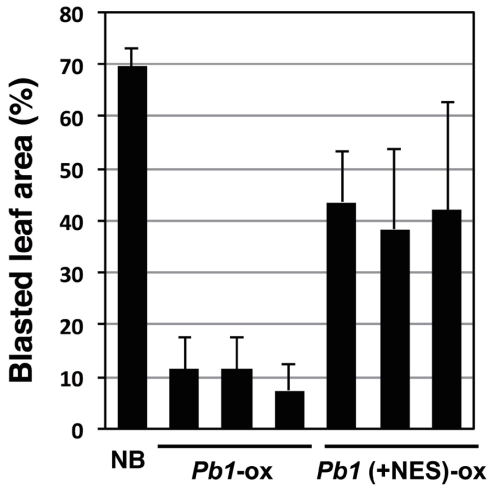

Fig. 3. Pb1 functions in the nucleus. A strong blast resistance was not observed in the transformant rice plants that overexpress Pb1 added with nucleus exclusion signal (NES).

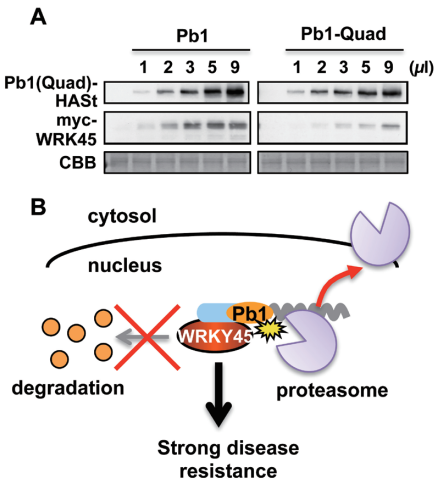

Fig. 4. Binding of Pb1 protects WRKY45 from proteasome degradation. (A) myc-WRKY45 was incubated with increasing amounts of Pb1-HASt or Pb1-Quad-HASt in wheat germ extracts, followed by co-immunoprecipitation with anti-HASt antibody.
(B) A model explaining the increase of blast.

Reference

1. Inoue H, Hayashi N, Matsushita A, Liu X, Nakayama A, Sugano S, Jiang CJ, Takatsuji H (2013) Blast resistance of CC-NB-LRR protein Pb1 is mediated by WRKY45 through protein-protein interaction ***Proceedings of the National Academy of Sciences USA*** 110(23):9577-9582