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Rice WRKY45 plays a crucial role in benzothiadiazole-inducible resistance to fungal blast and bacterial blight diseases

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Benzothiadiazole (BTH) is a so-called 'plant activator' and protects plants from diseases by 'priming' the salicylic-acid (SA) signalling pathway. Because of its characteristic action mechanism of 'priming', BTH exerts protective effects against a broad spectrum of diseases without major adverse effects on plant growth when applied at appropriate dosages. Assuming that a transcription factor plays a key role in the BTH-induced 'priming', we screened for BTH-inducible genes for transcription factors by using rice 44K oligoarray and identified *WRKY45* that upregulated within 3 h after BTH treatment. Overexpression of *WRKY45* in transgenic rice (*WRKY45*-ox) driven by the constitutive maize ubiquitin promoter dramatically enhanced resistance to blast disease (Fig. 1A), the devastating fungal disease worldwide. The *WRKY45*-ox rice was even more blast resistant than 'Sensho', which is known as a very highly blast resistant rice variety (Fig. 1C). *WRKY45*-ox rice plants were also highly resistant to bacterial blight diseases (Fig. 1B). Thus, *WRKY45*-ox rice is resistant to both fungal and bacterial diseases ('multi-disease resistance').

RNA interference–mediated knockdown of *WRKY45* (*WRKY45*-kd) compromised BTH-inducible resistance to blast disease, indicating that *WRKY45* is essential for BTH-induced defence responses. In a transient expression system, WRKY45 activated reporter-gene transcription through W-boxes, indicating that WRKY45 is a transcriptional activator acting through W-boxes. Epistasis analysis suggested that *WRKY45* acts in the SA signalling pathway independently of OsNPR1, a rice ortholog of Arabidopsis NPR1 (Fig. 2), which distinguishes *WRKY45* from any known Arabidopsis WRKY transcription factors. Examination of BTH inducibility in *WRKY45*- and *OsNPR1*-kd plants revealed that two defence-related genes, encoding a glutathione-S-transferase and a cytochrome P450, are regulated by *WRKY45*, but not by OsNPR1. On the other hand, *PR1b* and *PR1a* genes were regulated by OsNPR1, but not by WRKY45. These results are consistent with the apparent independence of the WRKY45- and OsNPR1-dependent pathways.

Thus, rice has a SA-signalling pathway that is substantially different from the Arabidopsis counterpart. *WRKY45*-ox rice plants grew without severe growth defects in our greenhouse (Fig. 3). In these plants, defence reactions were not activated as revealed by the absence of *PR*-gene expression (Fig. 3). Thus, *WRKY45*-ox rice appears to be 'primed' for defence responses. However, *WRKY45*-ox rice showed substantial growth retardation accompanied by *PR*-gene expression when grown in a growth chamber (Fig. 3), suggesting that some environmental factor (s) triggered defence reactions by acting downstream of *WRKY45* transcription (Fig. 2). The high degree of multi-disease resistance accompanied by minor growth retardation due to overexpression of *WRKY45* makes this gene a promising tool for developing practically useful multi-disease resistant rice by a transgenic approach. To achieve this goal, however, the environment-dependent growth retardation has to be overcome by optimizing the transgene expression.

Reference

Shimono M, Sugano S, Nakayama A, Jiang C-J, Ono K, Toki S, Takatsuji H (2007) Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. *Plant Cell*, **19**(6):2064-2076.

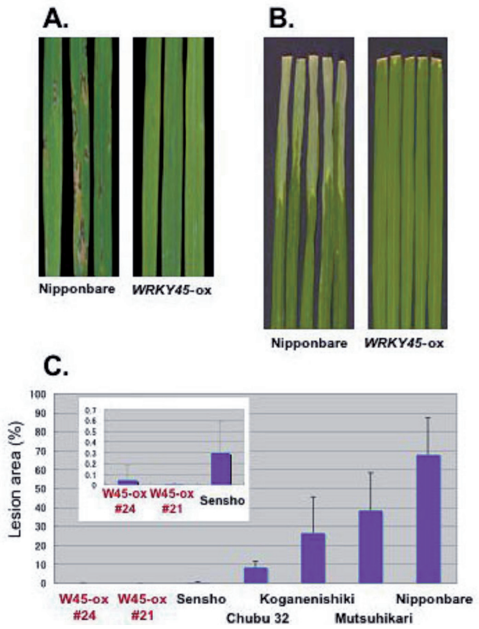

Fig. 1 Disease resistance of rice by overexpression of *WRKY45*
(A and B) Disease resistance of *WRKY45*-ox rice to fungal blast (A) and bacterial blight (B) diseases.
(C) *WRKY45*-ox rice is even more blast resistant than the highly blast resistant rice variety 'Sensho'.

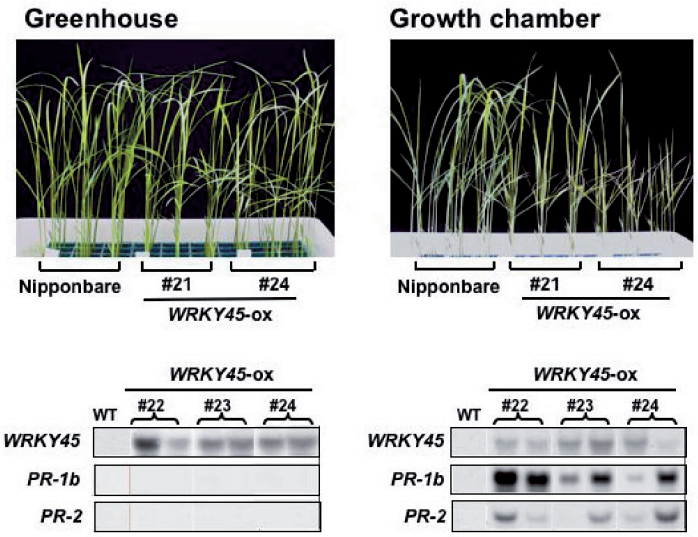

Fig. 2 SA signalling pathways in Arabidopsis and rice
In Arabidopsis, downstream of SA is largely controlled by NPR1. In rice, the SA pathway separate into WRKY45- and OsNPR1-dependent pathways. An environmental factor(s) acts downstream of WRKY45 and OsNPR1 to trigger defence-gene expression.

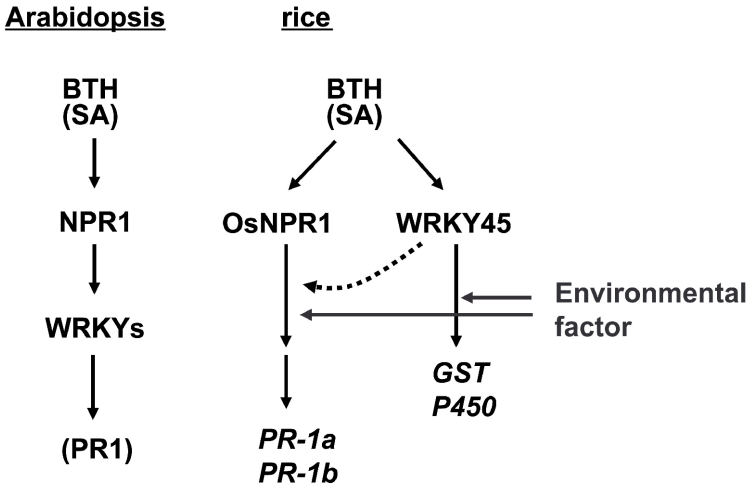

Fig. 3 Growth and *PR*-gene expression in *WRKY45*-ox rice plants under different growth conditions
Greenhouse-grown *WRKY45*-ox rice showed only minor growth retardation, whereas, it was much more severe in growth-chamber-grown *WRKY45*-ox rice. Expression of *PR1b* and *PR1a*, as markers of defence reactions, was undetectable in greenhouse-grown but highly detected in growth-chamber-grown *WRKY45*-ox rice.