



Breeding plant broad-spectrum resistance without yield penalties

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A central goal of crop improvement is to breed varieties with broad-spectrum resistance (BSR) to pathogens, but most of the major resistance (R) genes identified to date confer race-specific resistance to their adapted pathogens. Although these R genes are effective for a specific pathogen, their durability in the field is typically short due to mutations in the pathogen population that overcome the resistance. An alternative strategy of incorporating multiple R genes against different pathogens into elite cultivars is time-consuming and technically challenging, and usually results in a yield penalty as the crop diverts energy to implementing disease resistance (1). Many pathogens infect rice (*Oryza sativa*), the staple food crop of over half of the world's population. Among them, the fungal pathogen *Magnaporthe oryzae* and the bacterial pathogen *Xanthomonas oryzae* pv *oryzae* (*Xoo*) are two of the most destructive and can cause devastating yield losses in most rice-growing countries (2). Over the past two decades, many rice R genes have been identified, but none confers resistance to both pathogens. Although manipulating the expression of several defense-responsive genes, or genes in defense signaling pathways, has led to BSR (3, 4), few such genes have been successfully deployed in rice production for disease control. In PNAS, Zhou et al. (5) report the identification of the *broad-spectrum resistance Kitaake-1* (*Bsr-k1*) gene, which negatively regulates BSR, and the *bsr-k1* allele, which confers nonspecific BSR to both *M. oryzae* and *Xoo* without a yield penalty.

The resistant *bsr-k1* mutant was identified from an ethylmethane sulfonate-treated mutant population of the japonica cultivar Kitaake that had been inoculated with seven Kitaake-compatible *M. oryzae* isolates in the field (5). In addition, the *bsr-k1* mutant conferred enhanced resistance to 10 Kitaake-compatible *Xoo* isolates. Map-based cloning identified *Bsr-k1*, and further analysis showed that *Bsr-k1* gene expression is not induced by *M. oryzae* infection and the BSR-K1 protein localizes in the cytoplasm.

The disease resistance phenotype of the *bsr-k1* mutant results from a mutation that causes early termination of a tetratricopeptide repeat (TPR)-containing protein

(5). Some TPR-containing proteins are known to be involved in mRNA metabolism (6); therefore, Zhou et al. (5) performed in vitro RNA immunoprecipitation assays and found that the BSR-K1 protein binds to the mRNAs of the *phenylalanine ammonia-lyase* (*PAL*) genes and promotes mRNA turnover in rice (Fig. 1A). In contrast, the truncated *bsr-k1* protein (1–276 aa) in the *bsr-k1* mutant does not bind to *OsPAL* mRNAs (Fig. 1B). Consistent with the RNA binding results, overexpression of *OsPAL1* in transgenic rice enhances resistance to *M. oryzae* and causes an increase in lignin content. Together, these results demonstrate that BSR-K1 negatively regulates BSR by modulating turnover of *OsPAL* mRNAs.

PAL proteins are key enzymes in the phenylpropanoid pathway involved in the biosynthesis of lignin and flavonoids, and they contribute to disease resistance (7). In rice, there are nine members of the *PAL* gene family (8). Importantly, four of them (*OsPAL1–4*) are colocalized with the major resistance quantitative trait locus against *Rhizoctonia solani* and *Xoo* in the rice genome (8), and most *OsPALs* genes are induced by *M. oryzae* (9). The *ospal4* mutant shows increases susceptibility to three rice pathogens (*R. solani*, *Xoo*, and *M. oryzae*) (8), and the *OsPAL06* knockout mutant displays increased susceptibility to *M. oryzae* (9). These results demonstrate the importance of the *OsPAL* genes in BSR against diverse pathogens in rice. Similarly, Zhou et al. (5) found that the *bsr-k1* mutant has higher transcript levels of *OsPAL1–OsPAL7* than does Kitaake and that *OsPAL1* overexpression lines have increased resistance to *M. oryzae*. However, unlike *OsPAL4* and *OsPAL06* (8, 9), suppression of *OsPAL1* does not compromise resistance to *M. oryzae* in the *bsr-k1* mutant or Kitaake background (5), probably due to function redundancy with other *OsPAL* genes.

Although dozens of BSR genes have been identified in crop plants (3), few of them have been used extensively in crop production due to the consequent yield penalty (1, 10). For example, the *mlo* gene of barley has an average yield penalty of 4.2% (11). Interestingly, the *bsr-k1* mutation does not have clear adverse effects on major agronomic traits, compared with wild-type

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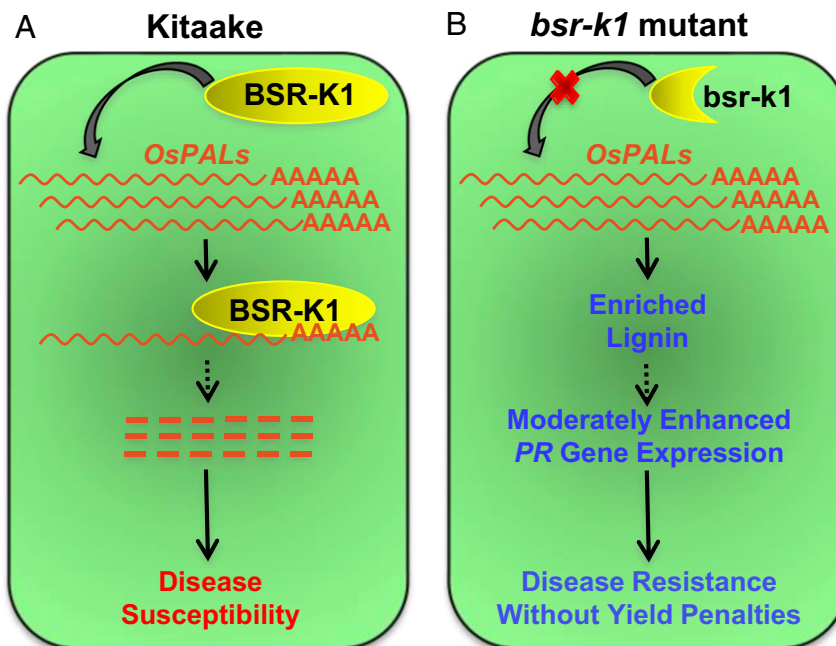


Fig. 1. Model for BSR-K1-mediated disease resistance. (A) In the wild-type Kitaake plants, BSR-K1 proteins bind to the mRNA of *OsPAL* genes and promote their turnover, leading to disease susceptibility. (B) In the *bsr-k1* mutant plants, the truncated *bsr-k1* protein cannot bind to the *OsPAL* mRNAs. The accumulation of *OsPAL* transcripts can increase the levels of secondary compounds such as lignin, which may moderately enhance *PR* gene expression and increase disease resistance without a yield penalty.

Kitaake, when growing in rice fields with or without high disease pressure. Furthermore, Zhou et al. (5) transferred the *bsr-k1* allele into three elite rice cultivars that are popular restoring lines for hybrid rice in Southwest China. These *bsr-k1*-containing cultivars showed similar yields to those of their parental lines in three field tests at two locations.

Recently, the same team identified a natural allele of the transcription factor gene *Bsr-d1* in rice that confers BSR to *M. oryzae*. A single-nucleotide mutation in the promoter of the *bsr-d1* gene results in the binding of the repressive MYB transcription factor, leading to an inhibition of H_2O_2 degradation and enhanced resistance (12). The identification of the *bsr-k1* allele provides another candidate gene for BSR breeding in rice.

TPR-containing proteins play central roles in the biogenesis of the photosynthetic apparatus and plant development (6). For example, the TPR-containing protein CGL71 participates in the assembly of photosystem I in *Chlamydomonas reinhardtii* (13). The chloroplast-localized TPR-containing proteins SG1 and WTG1 are required for chloroplast development in *Arabidopsis* (14, 15). TPR-containing proteins are also known to be involved in plant immunity. *Suppressor of rps4-RLD* (*SRFR1*), encoding a TPR-containing protein, modulates resistance to the bacterial pathogen *Pseudomonas syringae* pv. tomato strain DC3000 expressing *avrRps4* (16). *SRFR1* negatively regulates the accumulation of multiple R proteins, including SNC1, RPS2, and RPS4. Interestingly, *SRFR1* directly interacts with another TPR-containing protein, SGT1, which is required for the function of a number of R proteins (17). These results suggest that *SRFR1* associates with SGT1 to suppress R gene-mediated disease resistance. Although BSR-

K1 does not interact with the rice defense complex, including OsSGT1, as shown in this study (5), it remains to be determined whether BSR-K1 associates with any R protein that recognizes factors from *M. oryzae* or *Xoo*. Another possibility is that BSR-K1 protein may act as a susceptibility locus targeted by *M. oryzae* and/or *Xoo*.

This intriguing study establishes a link between a TPR-containing protein and *PAL* mRNA turnover in the context of plant BSR (Fig. 1); it also suggests a new strategy to breed durably resistant plants. However, the results also suggest several important areas of future investigation. First, the mechanism by which the BSR-K1 protein preferentially binds to different *OsPAL* mRNAs and promotes their turnover during pathogen infection is not known. Second, the relationship between BSR-K1 and R proteins, the OsRac1-mediated defense, and other defense signaling components needs to be elucidated. Third, the location in the cytoplasm where BSR-K1 binds to *OsPAL* mRNAs warrants study. Fourth, it is unclear whether, and how, the higher level of lignin content directly induces the *pathogenesis-related* (*PR*) gene expression in the *bsr-k1* mutant. Finally, an important question is whether moderate overexpression of *OsPAL1*, *OsPAL2*, *OsPAL3*, and *OsPAL4* in rice leads to BSR without a yield penalty. Addressing these questions will result in deeper mechanistic understanding of BSR-K1-mediated BSR, and ultimately result in better strategies for breeding durable resistance in crops.

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