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A LARGE SCALE FORWARD SCREENING IN RICE USING INSERTION MUTANT LINES TO DISSECT SIGNALING PATHWAY IN DISEASE RESISTANCE

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Plants have surveillance and defense response systems to protect themselves from pathogen attack. To date, some components that regulate the fundamental aspects of *R* gene-mediated defense responses have been isolated, however, the molecular mechanism of signal transduction after recognition of pathogen challenge is still unclear. In rice, a large-scale genetic screening for disease resistance has not been carried out, yet. The rice retrotransposon *Tos17* is one of a few active retrotransposons in plants and a useful tool as an endogenous insertional mutagen. In order to identify novel factors required for disease resistance, we applied rice lines mutagenized with *Tos17* to a forward screen using rice blast fungus *Magnaporthe grysea*. As the first screening, we inoculated 43,000 potential mutant lines with the incompatible race of the fungus containing *avrPish* recognized by a cognate *R* gene *Pish*. We have identified several lines that diminish the *Pish*-dependent resistance. The analysis of *Tos17* flanking sequences revealed that some of them have *Tos17* insertions in *Pish* gene itself, and other mutants decreased resistance against the fungus without mutation in *Pish*. The analysis of these mutants helps us to understanding the signal transduction mechanism of defense.

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THE ROLE OF THE NB-ARC DOMAIN IN R PROTEIN ACTIVATION AND THE ASSOCIATION OF R PROTEIN CHAPERONES

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Resistance (R) proteins in plants mediate pathogen recognition and subsequent induction of an immune response. As models we study the tomato R proteins I-2 and Mi-1. I-2 confers resistance to the fungal pathogen *Fusarium oxysporum* while Mi-1 confers resistance to root-knot nematode species, whitefly, and some biotypes of potato aphid. Both R proteins belong to the NB-LRR family. R proteins from this class contain a central NB-ARC domain (consisting of three subdomains, i.e. NB-ARC1-ARC2), and an N-terminal leucine-rich repeat (LRR) domain. Activation of R proteins by pathogen recognition often leads to a hypersensitive response (HR). Induction of HR can also be accomplished by introducing autoactivating mutations in an R protein. We provide a mutational analysis of several conserved motifs of the NB-ARC domain to obtain autoactivating mutations. The MHD motif located in the ARC2 subdomain is of particular interest, since mutation in this motif has been shown in various R proteins to result in autoactivation. Furthermore, we analysed the interaction of R proteins with (co-) chaperones by yeast two-hybrid analyses (I-2) and co-affinity purification with tagged R protein (Mi-1).

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FUNCTIONAL COOPERATIVITY BETWEEN PRF-PTO HETERODIMERS IN HOST RESISTANCE OF TOMATO AGAINST *PSEUDOMONA SYRINGAE*.

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Proteins of the STAND family containing conserved nucleotide binding (NB) domains are involved in many immunological processes such us the inflammatory response, programmed cell death, and specific resistance against plant pathogens. Members of this class often undergo oligomerization events during activation. The tomato proteins Pto and Prf coordinate the immune response elicited by the effector proteins AvrPto or AvrPtoB of *Pseudomonas syringae*, including hypersensitive cell death (HR). Pto is a kinase that interacts with these effector proteins, while Prf belongs to the NB-leucine rich repeat family (NB-LRR, subfamily of STAND), and interacts constitutively with Pto through a novel N-terminal domain. We show here that Prf forms homomultimers on transient expression in *N. benthamiana*, and indirect self-association of Pto mediated by a Prf bridge. Assays carried out with Pto and Prf gain- and loss-of-function mutants confirm the interactions, and demonstrate functional cooperativity between the individual Pto-Prf heterodimers within the complex.

PS 1-85

***OS*WRKY45 PLAYS A CRUCIAL ROLE IN BENZOTHIADIAZOLE-INDUCIBLE BLAST RESISTANCE IN RICE**

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Benzothiadiazole (BTH) is a 'plant activator' and protects plants from diseases by activating the salicylic-acid (SA) signaling pathway. By microarray screening, we identified BTH- and SA-inducible WRKY transcription factor (TF) genes. Overexpression of one of them, *OsWRKY45*, in rice markedly enhanced resistance to rice blast fungus. RNAi-mediated knockdown of *OsWRKY45* compromised BTH-inducible resistance to blast disease, thus indicating that *OsWRKY45* is essential for BTH-induced defense responses. In a rice protoplast transient expression system, *Os*WRKY45 activated transcription of a *luciferase* reporter gene through W-boxes, indicating that *Os*WRKY45 is a transcriptional activator. Epistasis analysis indicated that *Os*-WRKY45 acts in the SA signaling pathway apparently independently of NH1, a rice ortholog of *Arabidopsis* NPR1, which distinguishes *OsWRKY45* from known *Arabidopsis* WRKY TFs. We found two defense-related genes, encoding a glutathione-S-transferase and a cytochrome P450, that are regulated downstream of *OsWRKY45* by characterizing *OsWRKY45*-knockdown plants. These genes were not regulated by NH1, consistent with the apparent independence of the *OsWRKY45*- and NH1-dependent pathways. Of particular interest, defense-gene expression in *OsWRKY45*-overexpressed rice plants varied with growth conditions. Similar growth-condition dependence was also observed in BTH-treated plants. These results suggest that some environmental factor(s) acts downstream of *OsWRKY45* transcription, leading to defense-gene expression. On the basis of these results, we propose a role of *OsWRKY45*