Breaking restricted taxonomic functionality by dual resistance genes

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N-B-LRR-type disease resistance (R) genes have been used in traditional breeding programs for crop protection. However, functional transfer of NB-LRR-type R genes to plants in taxonomically distinct families to establish pathogen resistance has not been successful. Here we demonstrate that a pair of Arabidopsis (Brassicaceae) NB-LRR-type R genes, RPS4 and RRS1, properly function in two other Brassicaceae, Brassica rapa and B. napus, but also in two Solanaceae, Nicotiana benthamiana and tomato (Solanum lyco persicum). The solanaceous plants transformed with RPS4/RRS1 confer bacterial effector-specific immunity responses. Furthermore, RPS4 and RRS1, which confer resistance to a fungal pathogen Colletotrichum higginsianum in Brassicaceae, also protect against Colletotrichum orbiculare in cucumber (Cucurbitaceae). Thus the successful transfer of two R genes at the family level overcomes restricted taxonomic functionality. This implies that the downstream components of R genes must be highly conserved and interfamily utilization of R genes can be a powerful strategy to combat pathogens.

Plants trigger innate immunity responses to pathogens via a two-layer surveillance system composed of pattern recognition receptors (PRRs) and nucleotide binding-leucine rich repeat (NB-LRR) proteins that are encoded by resistance (R) genes.1 PRRs recognize microbe-associated molecular patterns (MAMPs) at a plasma membrane, and NB-LRR proteins subsequently detect pathogen-derived effectors inside the cell. Although interfamily transfer of PRR-mediated disease resistance has been successful,2 no R genes have been successfully expressed in a different family, a phenomenon which has come to be known as restricted taxonomic functionality (RTF) of R genes.3 Heterologous expression of NB-LRR type R genes in a taxonomically distinct family triggers either no response or inappropriate auto-immunity responses, suggesting that the regulatory or signaling components associated with NB-LRR protein-based resistance are family specific.4

A pair of Arabidopsis thaliana (Brassicaceae) NB-LRR type R genes, RPS4 and RRS1, function together to confer disease resistance against two taxonomically distinct bacteria, Pseudomonas syringae pv. tomato DC3000, which produces the effector AvrRps4 (Pst-avrRps4), and Ralstonia solanacearum strains, which express the PopP2 effector5 (Fig. 1). To determine whether the RPS4/RRS1 R gene pair also functions in non-Brassicaceae plants, we generated transgenic Nicotiana benthamiana (Solanaceae) plants expressing RPS4 and RRS1 under control of their cognate promoters. We found that either of the two bacterial effectors, AvrRps4 or PopP2 produced in planta via Agrobacterium-mediated transient expression, induced cell death in N. benthamiana transformed with both R genes (RPS4+RRS1), but not in plants expressing only RPS4 or RRS1. Importantly, the

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transgenic *N. benthamiana* plants showed no significant constitutive expression of inducible defense-related genes, indicating that the conferred resistance is effector specific.

*R. solanacearum* causes bacterial wilt, a serious soilborne disease of many plants worldwide. Resistance lines are urgently needed, as natural resource for resistance is limited and soil fumigation has not been effective. Tomato plants (*Solanum lycopersicum*) transformed with *RPS4* and *RRS1* also conferred resistance to *R. solanacearum* expressing popP2, indicating that the conferred resistance is specific for the PopP2 effector. *Pst-avrRps4* causes bacterial speck on tomato, a disease characterized by defoliation, blossom blight and lesions on developing fruit. In *Arabidopsis*, the transgenic tomato plants exhibited resistance against the *Pst-avrRps4* pathogen, but showed no significant constitutive expression of inducible defense-related genes, indicating that the conferred resistance is specific for the AvrRps4 effector. Thus, *RPS4* and *RRS1* are functional in at least two solanaceous plants, *N. benthamiana* and tomato.

In *Arabidopsis*, the dual *RPS4*/*RRS1* genes also confer resistance to the fungal pathogen *Colletotrichum higginsianum*. *Colletotrichum* spp cause anthracnose disease in a wide range of host plants, including cucumber (*Cucumis sativus*, Cucurbitaceae). We generated transgenic cucumber plants expressing **RPS4**/**RRS1** and inoculated them with *Colletotrichum orbiculare*, which infects cucurbits. WT cucumber plants developed brown necrotic lesions surrounded by a yellow halo, a typical symptom of anthracnose disease. **RPS4**/**RRS1** plants were highly resistant, developing only small necrotic flecks at the inoculated sites, indicative of an active defense reaction. Transgenic plants grew normally and did not express inducible defense-related genes, suggesting that autoimmunity is not induced by **RPS4**/**RRS1** in cucumber. These data indicate that **RPS4**/**RRS1** recognize effectors common to *Colletotrichum* or detect some alteration of a host protein targeted by both strains.

Our study demonstrates that introduction of the two **RPS4** and **RRS1** overcomes RTF and suggests that the downstream components of the **R** genes are highly conserved. It is likely that **R** gene-based immunity can be transferred to distantly related species once the right gene pair is identified. The number of known potential pairs of **R** genes from various plant species is increasing and we postulate that some of those pairs may also overcome RTF. In summary, this finding indicates that a new strategy can be used for creating pathogen-resistant vegetables and crops by using a previously unexploited resource of durable genetic resistance.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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