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Analysing the potential of antimicrobial and nematicidal proteins for improved plant disease resistance

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Plants have evolved an innate immune system that recognizes the presence of potential pathogens to trigger defensive responses. These responses include the enhanced expression of pathogenesis-related (PR) proteins such as PR-1, PR-2 (chitinases) and PR-3 (β-1,3-glucanases). However, attempts to protect plants via enhanced levels of PR proteins had limited success because pathogens secrete virulence factors (effectors) to suppres host immunity. To resolve this limitation, we used structurally different classes of defensive proteins from fungal origin to enhance plant disease resistance. In the first part of this research the hydrolytic enzymes, chitinase (Chi2) and endo-β-1,3-glucanase (Endo1) from the basidiomycete fungi *Coprinopsis cinerea* and *Coprinellus congregates* were used as antifungal proteins for plant protection. Coprinus species produce these hydrolytic enzymes to degrade their fruiting bodies for spore release. It is unlikely that pathogens have evolved effectors to inhibit these different classes of enzymes. Application of purified Endo1 to spores of the fungal plant pathogen *Botrytis cinerea* showed a direct inhibitory activity on fungal growth. However, the purified Chi2 did not show inhibitory activity tested alone or in combination with Endo1. Endo1-expressing plants were resistant to infection by *B. cinerea*. In contrast, Chi2-expressing plants showed similar susceptibility as wild type plants but they were more resistant to the phytopathogenic bacterium *Pseudomonas syringae*. Progeny of crossed plants with Endo1 and Chi2 proteins showed enhanced disease resistance to both tested pathogens.
In the second part of project, fungal lectins with nematocidal and insecticidal property were used to enhance plant resistance against plant-parasitic nematodes and herbivorous insects. *Coprinopsis cinerea* lectin 2 (CCL2), a fucoside-binding lectin, and *Marasmius oreades* agglutinin (MOA) were expressed in Arabidopsis plants. Transgenic plants were challenged with various pathogens and pests. The results revealed that CCL2-expressing plants are more resistant than wildtype plants to the cyst nematode *Heterodera schachtii*, to fungal pathogens including *B. cinerea*, and the phytopathogenic bacterium *P. syringae*. The mechanism of the CCL2-mediated enhancement of plant disease resistance depended on the fucoside-binding ability of the lectin as transgenic plants expressing a mutant version of CCL2 (Y92A), compromised in fucoside-binding, exhibited wild type disease susceptibility. MOA transgenic lines showed resistance to two plant-parasitic nematodes, the cyst nematode *H. schachtii* and the root-knot nematode *Meloidogyne incognita*. In addition, MOA-expressing plants showed improved resistance to the herbivorous diamondback moth *Plutella xylostella*. These results demonstrate a potential of fungal hydrolytic enzymes and defensive lectins in plant protection against agronomically important pathogens and pests.

Jury:
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