Specificity of Antifungal Pathogenesis-Related Proteins—Commentary

Sela-Buurlage MB, Ponstein AS, Bres-Vloemans SA, Melchers LS, Van den Elzen PJM, Cornelissen BJC (1993) Only specific tobacco (*Nicotiana tabacum*) chitinases and β-1,3-glucanases exhibit antifungal activity. Plant Physiol **101:** 857–863

This article bridged what started as two entirely different approaches to study proteins and enzymes involved in plant defenses against pathogens. One line originated in 1970, when it was discovered that additional proteins associated with systemic acquired resistance (SAR) were induced in tobacco (*Nicotiana tabacum*) plants that reacted hypersensitively to *Tobacco mosaic virus* (Van Loon and Van Kammen, 1970). Now well known as pathogenesis-related proteins (PRs), novel proteins that are induced in pathological or related situations, their functions remained enigmatic for more than 15 years. The second line developed from the notion that fungal attack, as well as ethylene, induced a chitinase in bean (*Phaseolus vulgaris*) leaves that could act as a potent inhibitor of fungal growth (Schlumbaum et al., 1986). The tobacco PRs were acidic, apoplastic proteins, whereas the bean chitinase was a basic, vacuolar protein. Except for a possible link through ethylene, no connections between these proteins were apparent. Then, serendipitously in the group of Bernard Fritig in Strasburg, some of the virus-induced tobacco PRs were found to possess endochitinase (Legrand et al., 1987) and endo-β-1,3-glucanase activity (Kauffmann et al., 1987) with potential fungal cell wall-hydrolyzing activity. These results provided a first explanation for why SAR was effective not only against viruses but also against other types of pathogens. In parallel, it was demonstrated in the lab of Thomas Boller in Basel that the nonhost pathogen *Fusarium solani* f. sp. *phaseolicola* induced both chitinase and glucanase activities in pea (*Pisum sativum*) pods and that combinations of the purified chitinases and glucanases could fully explain the inhibitory action of protein extracts from the pods on the in vitro growth of various pathogenic and saprophytic fungi (Mauch et al., 1988). These data clearly indicated that pathogen-inducible hydrolytic enzymes contribute to the inhibition of potential fungal pathogens and the reduction of disease.

Homology searches revealed that in tobacco the pathogen-inducible acidic, apoplastic PRs have basic, vacuolar counterparts that are regulated in a developmentally controlled and tissue-specific manner. Sela-Buurlage et al. (see reference above) tested the two acidic and two basic chitinases and the three acidic and one basic glucanases from tobacco on in vitro growth of *F. solani*, revealing that the acidic proteins were not, or were only slightly, inhibitory, while the basic forms were substantially more active. Moreover, combinations of the basic chitinase PR-3d and the basic glucanase PR-2e exhibited synergistic antifungal activity, with as little as 0.1 μg of protein causing complete inhibition of fungal growth. In a later study, transgenic tomato (*Solanum lycopersicum*) plants expressing both these proteins were found to be almost fully resistant against *Fusarium oxysporum* f. sp. *lycopersici* (Jongedijk et al., 1995). Such data have since been extended to other PRs and plant-pathogen combinations. These results provided evidence that the pathogen-inducible, apoplastic PRs do not contribute much to resistance against the inducer (but may still play a role in SAR against subsequent attack), whereas the constitutive, vacuolar counterparts may readily attack invading pathogens upon rupture of the tonoplast.

LITERATURE CITED

Jongedijk E, Tigelaar H, Van Roekel JSC, Bres-Vloemans SA, Dekker I, Van den Elzen PJM, Cornelissen BJC, Melchers LS (1995) Synergistic activity of chitinases and β-1,3-glucanases enhances fungal resistance in transgenic tomato plants. Euphytica **85:** 173–180

Kauffmann S, Legrand M, Geoffroy P, Fritig B (1987) Biological function of 'pathogenesis-related' proteins: four PR proteins of tobacco have 1,3-β-glucanase activity. EMBO J **6:** 3209–3212

Legrand M, Kauffmann S, Geoffroy P, Fritig B (1987) Biological function of pathogenesis-related proteins: four tobacco pathogenesis-related proteins are chitinases. Proc Natl Acad Sci USA **84:** 6750–6754

Mauch F, Mauch-Mani B, Boller T (1988) Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinase and β-1,3-glucanase. Plant Physiol **88:** 936–942

Schlumbaum A, Mauch F, Vögeli U, Boller T (1986) Plant chitinases are potent inhibitors of fungal growth. Nature **324:** 365–367

Van Loon LC, Van Kammen A (1970) Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. "Samsun" and "Samsun NN". II. Changes in protein constitution after infection with tobacco mosaic virus. Virology **40:** 199–211

Submitted by L.C. Van Loon
Plant-Microbe Interactions, Department of Biology, Faculty of Science, Utrecht University, 3508 TB Utrecht, The Netherlands

www.plantphysiol.org/cgi/doi/10.1104/pp.110.157859