

Defense Responses of *Hevea brasiliensis*Against Zoospores and Elicitin from Phytophthora palmivora

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Abstract

Phytophthora palmivora (Butler) is a pathogen of rubber tree (Hevea brasiliensis) which causes secondary leaf fall and black stripe leading to the decrease of rubber latex. It also causes root, stem and fruit rot on more than a hundred plant species, including pineapple, papaya, orange, tomato and durian. Rubber leaves produced a blue fluorescent phytoalexin (observed under UV light) after inoculation with zoospores of the fungus. The fluorescent compound was identified as scopoletin (Scp), hydroxycoumarin, on analyses by TLC and HPLC. The speed and extent of scopoletin accumulation measured by a spectrofluorophotometer were positively correlated with the resistant degree of rubber leaves to the pathogen. Scp exhibited a fungitoxic effect on the fungus with an 150 value of about 1.0 mM. Necroses detected on two rubber clones after spore inoculation were The lesions in the resistant clone (BPM-24) were black and did not obviously different. extend out of the treated zones as a consequence of hypersensitive cell death. In contrast, the necroses of the susceptible clone (RRIM600) were brown and expanded as a disease lesion. In addition, the accumulation of pathogenesis-related proteins or PR-proteins (β-1,3-glucanase and chitinase and lignin in were also associated with the resistance of rubber leaves to the fungus. Toxin from the culture filtrates of the fungus was purified by ammonium sulfate precipitation, followed by chromatography on DEAE-cellulose and Sephadex G-50. It was a protein of MW ca 10,000 daltons as determined by SDS-PAGE and stained with silver nitrate. The analyses of amino acid compositions and N-terminal sequences indicated that the toxin from this fungus is an elicitin, and named as palmivorein.

The proteins in elicitin family were also detected in other *Phytophthora* spp. such as *P. capsici*, *P. cactorum* and *P. parasitica*. The necrotic lesions caused by purified toxin were similar to those caused by spore inoculation. The accumulation of Scp, PR-proteins and lignin induced by toxin were highly produced in the resistant clone. Furthermore, the expression of PR-protein encoding gene (chitinase) was induced in the resistant clone after elicited with the elicitin. Therefore, the induction took place at the transcriptional level. The quantity of applied toxin was not only more precise than that of zoospore but it also induced defense reactions much more rapidly (within 24 hours). Therefore, the differences in characteristics of lesions, the levels of Scp, PR-proteins and lignin accumulation after zoospore inoculation and/or toxin treatment can be used as parameters in the selection of rubber clones resistant to *P. palmivora*.