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Changes in protein patterns of bean leaves after bean rust infection or application of elicitor

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Changes in patterns of soluble proteins of bean leaves after infection with bean rust (*Uromyces phaseoli*) were studied by electrophoresis in polyacrylamide pore gradient gels (4%-25% acrylamide).

Four newly formed *acidic* proteins not present in healthy leaves were found in extracts of inoculated leaves of the resistant, hypersensitively reacting variety (017). Their mol. wts were estimated as 17 000 (P_1), 27 000 (P_2), 33 500 (P_3) and 34 000 (P_4) (in the absence of SDS). The most prominent protein, P_1 , could be detected already 1 day and proteins P_2 - P_4 2-3 days after inoculation; all increased in amount up to 8 days after inoculation. All new proteins were restricted to the hypersensitively reacting tissue. Changes were also detected for soluble *basic* proteins. Five bands which were faintly visible in healthy leaves of the resistant variety increased dramatically in intensity between the 4th and 8th day after inoculation with the fungus.

Four new proteins (identical or very similar in size to those found in the resistant bean variety) were induced in non-inoculated leaves of the susceptible variety Favorit by infiltration of a polyglucan which acts as an elicitor of phytoalexin synthesis (1). Inoculation with the fungus 2 h after infiltration with the elicitor resulted in a successful infection, whereas an infiltration 3 days *before* inoculation caused total protection, correlated with the presence of the four new proteins.

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Induction of pathogenesis-related (b) proteins in Phaseolus vulgaris upon fungal or viral infection or after chemical injury

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Primary leaves of French bean (*Phaseolus vulgaris* L.) cvs Brittle wax or Immuna react with necrotic lesions to infection with either *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav. or alfalfa mosaic virus (AMV).

Electrophoresis in 10% polyacrylamide gels of the soluble leaf proteins present in extracts of leaves infected with *C. lindemuthianum* or AMV revealed three bands (p1, p2 and p3) which were not seen in extracts of healthy leaves. Foliar sprays with the growth regulator Aliette (Phosethyl-Al) at 2000 ppm (a.i) delayed the appearance of

necrotic local lesions induced by AMV on primary bean leaves, and reduced their final number. The delay in formation of necrotic lesions was correlated with a similar delay in the appearance of induced b-proteins.

Band p1, and bands p1, p2 and p3 were also observed when necrotic lesions were induced by treatment with mercuric chloride, or triphenylphosphite, respectively. There was a strong correlation between cell injury and induction of new proteins in bean leaves.

Ethylene-induced chitinase: is it a pathogenesis-related protein?

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Ethylene or an ethylene-releasing compound, 2-chloroethylphosphonic acid (ethephon) have been found to induce pathogenesis-related proteins (PRs) in tobacco leaves (1). Ethylene has also been found to induce a highly active endochitinase in many plants; for example, in bean leaves, chitinase activity increased 30-fold within 24 h of an ethylene treatment (2). In fully induced leaves, more than 1% of the total protein, or more than 5% of the protein in a crude extract obtained at pH 5, consisted of chitinase. The enzyme was purified to homogeneity; its apparent mol.wt obtained by SDS-polyacrylamide gel electrophoresis was 30 000. While no endogenous substrate for purified chitinase was found in the plant, the enzyme readily attacked cell walls of a potential pathogenic fungus, *Fusarium solani*, and acted as a lysozyme on bacterial cell walls. This led us to the hypothesis that chitinase functions as a defense against pathogens (2).

We were interested to know whether ethylene-induced chitinase has any relation to PRs. We found that purified bean chitinase, due to its high isoelectric point, did not enter a non-denaturing polyacrylamide gel upon electrophoresis at pH 8.3, which is generally used for the separation of PRs. Thus, chitinase, at least from bean leaves, is different from the PRs currently investigated.

On the other hand, experiments with pea pods infected with *Fusarium solani* and with tobacco leaves reacting hypersensitively to tobacco mosaic virus showed that chitinase is also induced in the course of a pathogen attack. Although an enhanced synthesis of ethylene accompanied the plant's response in both cases, stress ethylene formation was not a necessary condition for chitinase induction, at least not in pea pods: a treatment of pea pods with aminoethoxyvinylglycine (AVG), a specific inhibitor of ethylene biosynthesis, prevented stress ethylene formation but did not affect the induction of chitinase. Thus, chitinase is induced by pathogenesis independently of ethylene.

We conclude that chitinase, while being different from the PRs presently studied, is a pathogenesis-related protein in a broader sense.

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