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Bean-*Colletotrichum lindemuthianum* Compatible Interactions: Time Course of Plant Defense Responses Depends on Race

Aline Mahé, Jeanine Grisvard, Thierry Desnos, and Michel Dron

Laboratoire de Phytopathologie Moléculaire, Département de Biologie Moléculaire Végétale, Bâtiment 430, Université Paris Sud, 91405 Orsay Cedex, France.
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We investigated different compatible race-cultivar interactions between *Colletotrichum lindemuthianum* and bean to understand the role of the pathogen and the plant genetic background on the expression of symptoms, infection processes, and transcriptional activation of plant defense genes. Different races of *C. lindemuthianum* (1, 2, and 9) were used to inoculate one bean cultivar ($P_{12}S$). Races 1 and 2 displayed very similar patterns of pathogenicity (symptoms and infection process) and caused similar accumulations of four defense-related mRNAs (phenylalanine ammonia-lyase, chalcone isomerase, chitinase, and hydroxyproline-rich glycoprotein). In contrast, race 9 provoked symptoms more rapidly. This was correlated with a faster progression of the fungus and an earlier accumulation of the induced mRNA transcripts. No accumulation of the hydroxyproline-rich glycoprotein transcript was observed after infection with race 9. The interaction of two bean cultivars ($P_{12}S$ and EyS) with race 2 was further investigated. The two cultivars exhibited very similar behaviors after inoculation with race 2.

Additional keyword: defense mRNA induction.

Infection by pathogens causes a marked stimulation of the expression of a number of plant defense genes (Edwards *et al.* 1985; Dixon and Harrison 1990). Some of these genes, including genes involved in phytoalexin biosynthesis, are expressed locally at the infection site, whereas others such as chitinase and hydroxyproline-rich glycoprotein (HRGP) genes have a more systemic expression (Dixon and Harrison 1990; Bowles 1990).

These different defense responses have been extensively studied in the genetically controlled interaction of French bean with the fungal pathogen *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., the causal agent of the anthracnose disease. This interaction seems to follow the gene-for-gene hypothesis (Flor 1971). However, although resistance genes have been genetically identified in the host, the corresponding avirulence genes of the pathogen, an ascomycete, are only defined by the specific phenotype exhibited after infection of bean cultivars carrying differential resistance genes.

In bean, isoflavonoid secondary metabolites act as phytoalexins. These antimicrobial compounds are produced at the infection site (Dixon and Harrison 1990). Phenylalanine ammonia-lyase (PAL) catalyzes the first step in the phenylpropanoid pathway, which leads to lignins and flavonoid pigments in addition to isoflavonoids. Chalcone isomerase (CHI) catalyzes the second reaction of a branch pathway involved in the production of flavonoid pigments and isoflavonoids. Systemically induced defense reactions, such as chitinase (CHT) mRNA induction (Hedrick *et al.* 1988), have also been observed in bean in response to fungal infection. This hydrolase has been shown to degrade fungal wall components causing growth inhibition *in vitro* (Mauch *et al.* 1988) but also to favor the release of fungal wall fragments that might be active elicitors of plant secondary metabolites (Keen and Yoshikawa 1983). Induction of HRGP, an important structural component of plant cell walls, is another example of the systemic response to pathogen invasion (Corbin *et al.* 1987). It has been suggested that HRGPs restrict pathogen penetration by strengthening the wall and/or inhibiting the growth of microbes by agglutination (Bowles 1990).

Generally, temporal differences in the pattern of accumulation of defense gene transcripts between compatible (host-susceptible) and incompatible (host-resistant) interactions have been reported (Dixon and Harrison 1990). The experimental model often consisted of only one plant cultivar infected with two different races of the pathogen: a virulent race for the compatible interaction and an avirulent race for the incompatible interaction (Hedrick *et al.* 1988; Habereder 1989; Fritzemeier *et al.* 1987; Voisey and Slusarenko 1989; Dong *et al.* 1991). In these cases, a major question is whether there is an interference between two events: a response ascribed to a molecular recognition specified by the plant disease resistance gene and the pathogen avirulence gene that underlies race-cultivar specificity and a response to different non-specific reactions arising from fungal ingress when two different races are used (possible difference in the pathogenicity of these races).

To address this point, we have focused on a study of different compatible interactions involving one bean cultivar ($P_{12}S$) and three races of *C. lindemuthianum* (races 1, 2, and 9) (Charrier and Bannerot 1970), which all cause disease on this cultivar. In this paper, we have shown that these different pathogen races induced different time courses of PAL, CHI, CHT, and HRGP mRNA accumulation. To correlate the mRNA inductions with the

Address correspondence to M. Dron.

infection process, we also investigated the three compatible interactions at the cytological level. In contrast, no difference in the development of the fungus and in plant gene activation was detected in relation to the plant genetic backgrounds when two different compatible interactions involving one race of *C. lindemuthianum* (C12) and two different bean cultivars ($P_{12}S$ and EyS) were examined.

MATERIALS AND METHODS

Plant and fungal material. Bean cvs. $P_{12}S$ and EyS (*Phaseolus vulgaris* L.) susceptible to races 1, 2, and 9 of *C. lindemuthianum* were grown, and leaves were inoculated by spraying them with a suspension of conidia (10^7 spores per milliliter) as described previously (Mahé *et al.* 1992). Control plants were mock-inoculated with water and incubated under identical conditions. Seeds of $P_{12}S$ and EyS were provided by H. Bannerot and G. Fouilloux (INRA, Versailles, France). Races of *C. lindemuthianum* were supplied by F. Legrand and J. Tailler (laboratoire Cryptogamie, Orsay, France). Races 1 and 2 belong to the group of α races, and race 9 belongs to the group of γ races described previously by Charrier and Bannerot (1970).

Leaves were collected for cytological and molecular analysis every 8 hr until the leaves were completely rotten (up to 140 hr for inoculation with races 1 and 2 and up to 108 hr with race 9).

DNA probes. The plant defense gene probes, the bean cDNA clones coding for phenylalanine ammonia lyase ($pPAL_5$; Edwards *et al.* 1985), chalcone isomerase ($pCHI_1$; Mehdy and Lamb 1987), chitinase (pCHT; Hedrick *et al.* 1988), and hydroxyproline-rich glycoprotein (pHyp 4.1; Corbin *et al.* 1987) were gifts from C. J. Lamb (Salk Institute, San Diego, CA, U.S.A.).

The constitutive gene probe, the bean cDNA clone coding for the translation elongation factor EF-1α (pCHA0041; Axelos *et al.* 1989), was kindly provided by B. Lescure (Université Paul Sabatier, Toulouse, France).

The 1.1-kb *Pst*I insert fragment of the $pPAL_5$ clone, the 0.86-kb *Hin*dIII insert fragment of the $pCHI_1$ clone, the 0.65-kb *Eco*RI-*Hin*dIII insert fragment of the pCHT clone, the 0.7-kb *Hin*dIII insert fragment of the pHyp 4.1 clone, and the 1.1-kb *Eco*RI-*Bam*HI insert fragment of the EF-1α plasmid were labeled by primer extension with random hexanucleotide primers (Maniatis *et al.* 1989) and were used as probes.

Cytology. Pieces of fresh leaves were harvested at various times after inoculation, cut into 1-cm^2 fragments, cleared, and stained for cytological observation as described (Mahé *et al.* 1992).

RNA blot hybridization. To reduce plant-to-plant variation, four leaves, each from a different bean plant, were collected for each experimental time point, pooled, and frozen in liquid nitrogen.

Total cellular RNA was isolated from frozen tissues by phenol sodium dodecyl sulfate (SDS) chloroform extraction (Mahé *et al.* 1992). The RNA was quantified; we assumed an A_{260} of 1 for 40 μg/ml of solution.

For dot blot analyses, the RNA samples were denatured in boiling water for 5 min and cooled immediately to 4° C in ice. A 1/10 vol of 20× SSC (1× SSC contains 150 mM sodium chloride, 15 mM sodium citrate) was added. Dot blots were carried out by spotting 4 μl of denatured RNA (0.5, 0.2, or 0.1 μg) onto a nylon membrane (Hybond N^+, Amersham Corp., Arlington Heights, IL); four dots were performed for each RNA sample. RNA was cross-linked to the membrane by exposure to UV light (Germicide tubes TUV 6, Philips, Mahwah, NJ) for 5 min.

Prehybridization was in 2× SSC, 5× Denhardt's solution (1× Denhardt's solution is 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpolypyrrolidone) at 42° C for 5 hr. Hybridization was carried out at 42° C for 16–18 hr in 0.5% SDS, 5× Denhardt's solution, 100 μg/ml of salmon sperm DNA, 50% formamide, and 4× SSC for the PAL_5, CHI, CHT, and EF-1α probes and 2× SSC for the Hyp 4.1 probe. After hybridization, the filters were washed twice with 0.1× SSC, 0.5% SDS at room temperature for 30 min, and twice with 0.1× SSC, 0.1% SDS at 46° C for 30 min. Filters were then exposed to X OMAT-AR Kodak films (Rochester, NY) for 2–3 days at −80° C with an intensifying screen.

Hybridizations with the defense gene probes and EF-1α probe were performed successively on the same blot. Between each hybridization, the probes were removed by washing the filters twice in boiling 0.01× SSC and 0.5% SDS for 5 min and shaking at room temperature for 30 min.

Quantification and normalization. After hybridization of the RNA dot membranes with a specific probe coding for genes involved in plant defense responses and after probe stripping, the blots were rehybridized with a bean EF-1α probe that has been shown to be specific to plant RNA and constitutively expressed in our experimental conditions (Mahé *et al.* 1992). The quantification of the transcript levels was achieved by densitometric scanning of the autoradiograms (image analyser, pdi, Kontron, Milan, Italy). For each dot, the scanning value of the autoradiographic signal obtained by hybridization with a specific plant defense gene probe (PAL, CHI, CHT, and Hyp 4.1) was divided by the scanning value of the hybridization signal obtained with the EF-1α probe. This ratio was then multiplied by a constant value corresponding to the mean of scanning values measured for EF-1α during an infection kinetics. The resulting values expressed in arbitrary units (AU) were plotted as a function of infection time. In this way, data were normalized to a given amount of plant RNA (Mahé *et al.* 1992).

RESULTS

Patterns of defense responses after infection of a bean cultivar by race 1, 2, or 9 of *C. lindemuthianum*. Leaves of the bean cv. $P_{12}S$ were inoculated with spores of race 1, 2, or 9 of *C. lindemuthianum*.

Disease symptoms and fungal development. The kinetics of symptom development were similar during the interaction between the bean cv. $P_{12}S$ and race 1 or 2, but differed in the interaction with race 9, as summarized in Figure 1. The first symptoms to appear were small dark brown lesions on the veins (α), which became visible by 92 hr after infection by races 1 and 2 and 60 hr after infection

by race 9. They spread and resulted in a complete brownish coloration of the veins 16 hr later, whichever virulent race was examined (β). Watersoaked lesions and macerated host tissues appeared after an additional delay of 24 hr for the interactions with races 1 and 2 or after 16 hr with race 9 (δ). Thus, the timing of symptom development after infection with race 1 or 2, or with race 9, differed by 40 hr. Leaves inoculated with race 9 were completely rotten (δ) at the time when only small dark brown lesions (α) were visible on leaves infected with race 1 or 2 (Fig. 1).

The different steps of the development of *C. lindemuthianum* in bean tissues have been described by O'Connell *et al.* (1985) and are schematized in Figure 1. Conidia, or spores, germinate and differentiate to appressoria (a) on the surface of the leaves. After penetration of tissues from susceptible plants, intercellular primary hyphae emerge from vesicles formed in epidermal cells (b), develop in the infected cell (c), and colonize the adjacent cells. When the primary mycelium is well established (d), narrow secondary hyphae branch out of the primary hyphae and invade the host cells (e). The cytological analysis of the infection process in the three interactions investigated in this study (Fig. 1) shows that races 1 and 2 developed in leaves of bean cv. $P_{12}S$ with roughly similar kinetics, whereas the progression of race 9 was more rapid. Races 1 and 2 entered into the bean cells after a period of 28 hr (a, Fig. 1), whereas penetration of infection pegs from race 9 occurred slightly earlier (at 20 hr). Primary hyphae were developed and had colonized several cells 24 hr earlier (d, Fig. 1) in leaves challenged with race 9, and in these leaves fungal mycelium had extensively invaded the tissues at 92 hr. At this time, secondary hyphae had not yet appeared in leaves infected with race 1 or 2 (e, Fig. 1).

Changes in plant defense gene mRNA amounts after infection. The expression of four plant defense genes encoding PAL, CHI, CHT, and Hyp 4.1 was investigated by RNA gel blots and dot blot hybridizations of total cellular RNA.

The RNA gel blot hybridization experiments revealed that each probe specifically hybridized with single RNA species of 2.6, 1.1, 1.2, and 2.4 kb for PAL, CHI, CHT, and Hyp 4.1 probes, respectively (data not shown). These mRNA lengths correspond to those already determined in bean (Bell *et al.* 1986; Mehdy and Lamb 1987; Hedrick *et al.* 1988; Sauer *et al.* 1990). Because these control experiments attested the quality of the RNA preparations and the accuracy of the hybridization conditions, changes in mRNA abundance were estimated by dot blot analysis.

As already described (Bell *et al.* 1986), the PAL mRNA was present at a very low, almost undetectable, basal level in the mock-infected plants, whereas it accumulated after infection as shown in Figure 2. The kinetics of appearance of PAL transcripts followed a similar pattern in bean leaves infected either with race 1 or 2; an early, very transient, and low increase in PAL mRNA was observed at 28 hr after inoculation. A second more pronounced induction of PAL mRNA took place first slowly by 76–92 hr after inoculation, then more rapidly to reach a maximum

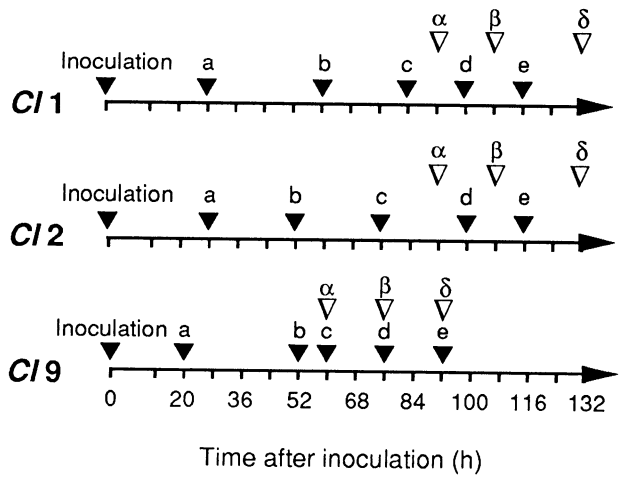

Fig. 1. Time course of lesion spread and pathogen development during the compatible interactions of bean cv. $P_{12}S$ with *Colletotrichum lindemuthianum* race 1, 2, or 9 (Cl1, Cl2, or Cl9). Visual symptoms (△) observed on the surface of the leaves are α, small brown lesions on veins; β, brownish color of the veins; δ, watersoaked lesions and macerated host tissues. Cytological observations (▲) are a, appressorium; b, primary hyphae emerging from infection vesicles; c, developed primary hyphae in the infected cell; d, extensive development of primary hyphae; e, well-developed secondary hyphae.

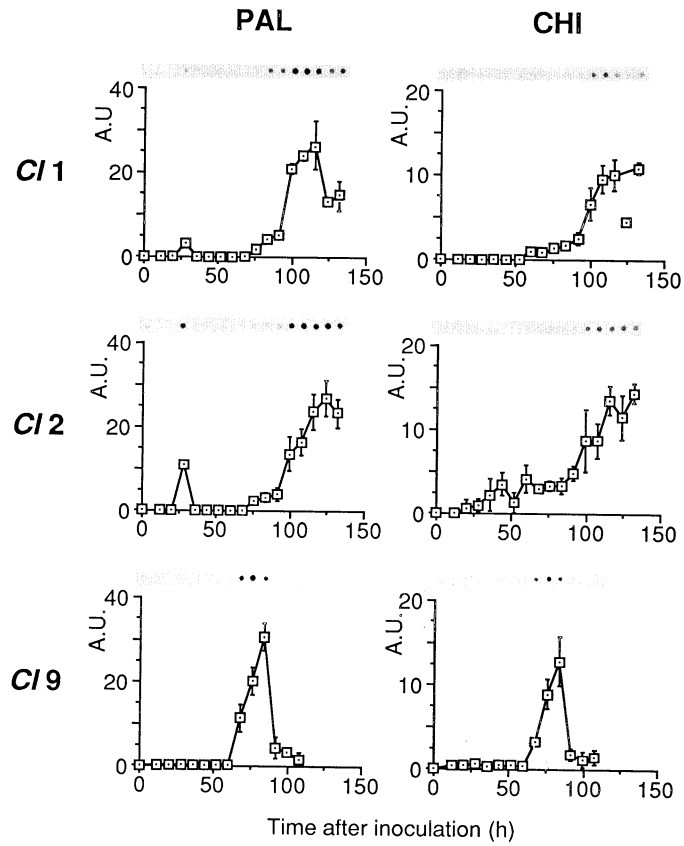

Fig. 2. Time course of PAL (phenylalanine ammonia-lyase) and CHI (chalcone isomerase) mRNA inductions in bean cv. $P_{12}S$ infected with *Colletotrichum lindemuthianium* race 1, 2, or 9 (Cl1, Cl2, or Cl9). Total bean RNA isolated from leaves, harvested at the indicated times after infection, was spotted onto nylon membranes and hybridized to the ^{32}P-labeled inserts of $pPAL_5$ and pCHI bean cDNA clones as indicated on the figure. The mRNA levels were quantitated as described in text. Bars represent two times the standard deviation from four replicates. The autoradiographs on top of each graph show an illustration of RNA dot blot analysis.

between 116 and 124 hr for infection with race 1 and between 124 and 132 hr for infection with race 2. When the inoculation was performed with race 9, the early sharp peak was not detected. In this later case, the PAL mRNA induction started 60 hr after inoculation, increased dramatically, reached a maximum at 84 hr after inoculation, then rapidly decayed to almost the basal level. The kinetics of PAL mRNA induction after infection with race 9 was, thus, very different from that observed after infection with races 1 and 2. The main accumulation of PAL transcripts occurred earlier (at 84 hr instead of 116 hr) and was shorter in time (lasting only 40 hr instead of 75 hr). Furthermore, the early PAL mRNA induction that accounted for 10 and 40% of the main induction (percentage of the magnitude of the peaks) for the interactions with races 1 and 2, respectively, was not detected during the interaction with race 9.

As already observed for PAL mRNA induction, the kinetics of CHI and CHT transcript accumulation roughly exhibited the same pattern when bean leaves were infected by either race 1 or 2. CHI and CHT mRNA levels were barely detectable in the first hours after inoculation, then they increased and were maintained at high levels until the later stages of the infection as observed in Figures 2 and 3. During the compatible interaction between bean and race 9, the timing and the profile of CHI and CHT mRNA inductions (maximal level at 84 hr) were similar to that observed for the accumulation of the PAL transcripts. Again, induction of CHI and CHT mRNAs occurred earlier and extended over a shorter period when infection was obtained with race 9 as compared with race 1 or 2.

No noticeable differences were observed in the overall pattern of Hyp 4.1 mRNA induction during the interaction between $P_{12}S$ and race 1 or 2 (Fig. 3). The Hyp 4.1 transcripts started to accumulate by 75 hr after inoculation, reached a maximum level between 116 and 124 hr, then subsequently decreased. In contrast, during the infection of bean leaves with race 9, no marked and significant change could be observed in the level of the Hyp 4.1 transcript.

Comparative study of the race 2 interaction with two different susceptible bean cultivars. The two bean cvs. $P_{12}S$ and EyS were challenged with the virulent race 2 of *C. lindemuthianum*. Comparative studies of these two compatible interactions did not reveal significant differences either in the disease symptom development or in the fungus progress within the host cells as shown in Figure 4.

Similarly, the analysis of the kinetics of PAL, CHI, CHT, and Hyp 4.1 mRNA induction did not present any obvious differences between the two interactions. Therefore, only data with PAL and Hyp 4.1 as probes are presented in Figure 5. They showed that PAL mRNA accumulation was biphasic, and Hyp 4.1 transcripts were induced in leaves of EyS infected with race 2. The early PAL induction represented 10% of the maximum level of the main induction in this case.

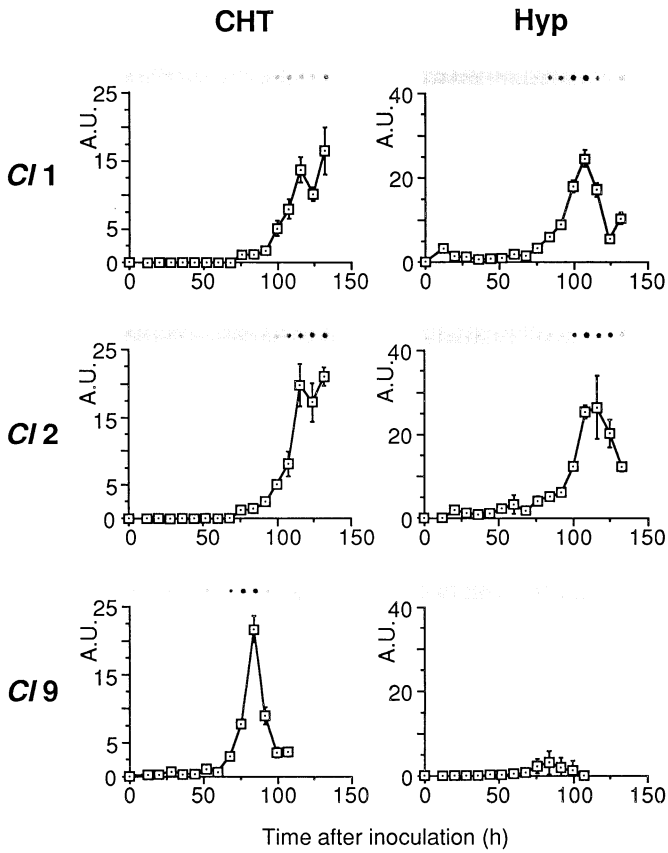

Fig. 3. Time course of CHT (chitinase) and Hyp 4.1 (hydroxyproline-rich glycoprotein) mRNA inductions in bean cv. $P_{12}S$ infected with *Colletotrichum lindemuthianum* race 1, 2, or 9 (Cl1, Cl2, or Cl9). The legends are the same as in Figure 2, except that the probes were from pCHT and pHyp 4.1 bean cDNA clones.

DISCUSSION

Consistent with earlier reports (Bell *et al.* 1986; Corbin *et al.* 1987; Hedrick *et al.* 1988), *C. lindemuthianum* infection of susceptible plants resulted in the accumulation of plant defense mRNAs.

The maximum of accumulation of PAL, CHI, CHT, and Hyp 4.1 mRNAs occurred at the time when primary hyphae had invaded many cells. At these times, spreading anthracnose lesions were visible on the leaf surface. Some signals may be produced during a particular step of the fungal growth arising from the plant cells, the fungus, or from both organisms. The perception of these signals would lead to an extensive development of fungal primary hyphae and correlatively to switch on some plant defense genes.

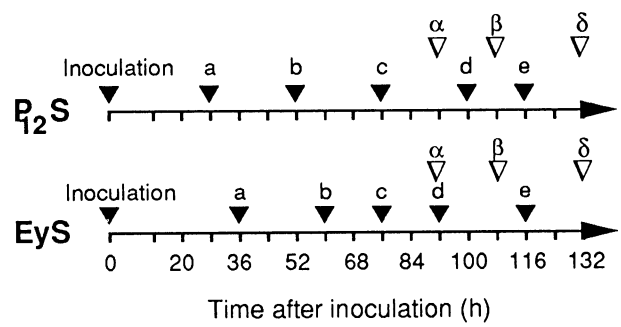

Fig. 4. Time course of lesion spread and pathogen development after challenge of bean cvs. $P_{12}S$ and EyS with *Colletotrichum lindemuthianum* race 2. The legends are as presented for Figure 1.

Effect of the host genetic background. In the present report, no significant differences were observed in the pattern of plant defense response for bean cvs. $P_{12}S$ and EyS infected with race 2 in the appearance of symptoms, in timing of the fungus progression, or in the time course of PAL, CHI, CHT, and Hyp 4.1 mRNA inductions. Although these two cultivars have phenotypic differences in plant height, hypocotyl thickness, leaf size, and presence of anthocyanins, they might be quite closely related genetically. Few polymorphisms have been detected between these two cultivars with restriction fragment length polymorphism (RFLP) analysis (unpublished data).

Differences in the response of cell suspensions of the bean cvs. Canadian Wonder and Immuna to elicitor have been reported (Liang *et al.* 1989). In studying incompatible interactions, O'Connell and Bailey (1988) showed that the development of a given race of *C. lindemuthianum* was arrested more or less rapidly in different bean cultivars. To determine whether the induction of the plant defense reactions in our model system might be dependent on the plant genetic background, we will select suitable cultivars by screening for differences in the timing of symptom development after they are challenged by different *C. lindemuthianum* isolates.

Effect of the pathogen genetic background. The overall scheme of fungal development was similar in the three interactions. However, race 9 developed and colonized bean tissues more rapidly than races 1 and 2. The visual symptoms were correlated with the same stage of the fungus development in the three cases, that is, after the production of extensive primary mycelium (c, α, Fig. 1). The extensive development of these hyphae was followed by the appearance of a brownish color of the veins in every case studied (d, β, Fig. 1). During infection by race 9, watersoaked lesions became visible in correlation with the development of secondary hyphae; in contrast, a delay of 16 hr was observed between these two events in infection by race 1 or 2 (f, δ, Fig. 1). Finally, the leaves were completely rotten 40 hr earlier when challenged with race 9 than when inoculated with race 1 or 2.

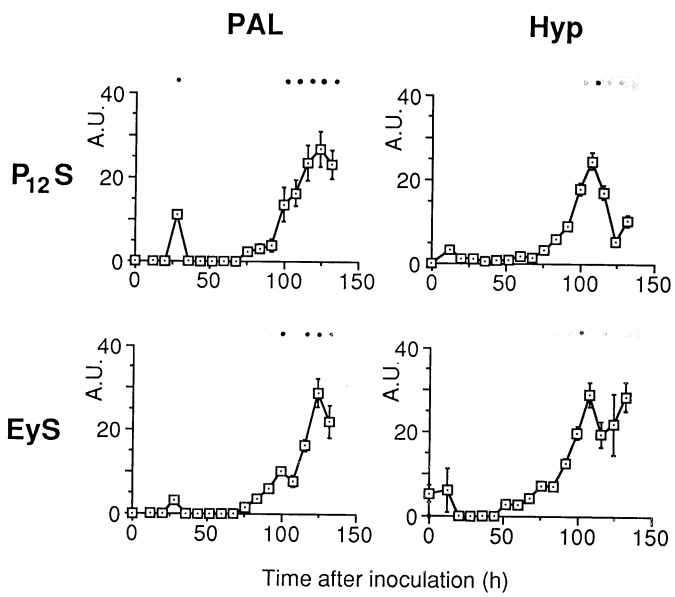

Fig. 5. Time course of PAL (phenylalanine ammonia-lyase) and Hyp 1.4 (hydroxyproline-rich glycoprotein) mRNA inductions in bean cvs. $P_{12}S$ and EyS infected with *Colletotrichum lindemuthianum* race 2. The legends are as presented for Figure 2. The probes were from $pPAL_5$ and pHyp 4.1 bean cDNA clones as indicated on the top of the curves.

The plant defense genes studied in this report were also activated differently in response to infection by the different races of the pathogen. According to the race, different parameters were affected. Timing of induction was affected; PAL, CHI, and CHT mRNAs were induced 32 hr earlier when plants were infected with race 9 than with races 1 and 2. Length of induction was affected; PAL (main induction), CHI, and CHT mRNAs were accumulated for a shorter time, approximately 40 hr, when plants were infected with race 9 compared to more than 75 hr when they were infected with race 1 or 2. The nature of gene transcript accumulation was affected as well.

An early minor peak of PAL mRNA accumulation was observed only in the interactions with races 1 and 2. Although detected at only one time point, it appears to be significant because it was reproducible in different experiments involving different interactions ($P_{12}S$-race 1, $P_{12}S$-race 2, and EyS-race 2), and it was not observed after a mock infection. It is specific to PAL transcript because no induction of CHI, CHT, and Hyp 4.1 was observed by this time. The bimodal induction kinetics of PAL mRNA may be attributable to the differential activation of multiple genes. To gain more detailed insight into this induction, further analyses with specific PAL gene probes should be performed. In terms of fungal development, the early PAL induction occurred at the time of the differentiation of the fungal infection peg. During the interaction between cv. $P_{12}S$ and race 9, the first PAL transcript accumulation was not revealed (Fig. 2), just as in the interaction between bean cv. Kievitsboon Koekoek and *C. lindemuthianum* race γ (Bell *et al.* 1986). This could be either because the early PAL peak did not occur at all during these interactions or because the induction was so transient that none of the samples matched this induction. Difference in Hyp 4.1 mRNA induction was also observed among the races. The infection with race 9 did not affect the Hyp 4.1 mRNA steady-state level. This result was unexpected because Corbin *et al.* (1987) described a strong induction of Hyp 4.1 mRNA during a compatible interaction between bean cv. Kievitsboon Koekoek and a race γ of *C. lindemuthianum*. In our case, race 9 is also a γ race (Charrier and Bannerot 1970). So, the observed discrepancy may be due to the different backgrounds of the infected cultivars (Kievitsboon Koekoek versus $P_{12}S$). However, the question of the relatedness of the different γ isolates of *C. lindemuthianum* can also be raised. This observation illustrates that in bean infected with *C. lindemuthianum* the Hyp 4.1 gene is not coordinately regulated with the other genes investigated in this study; this was already suggested by Showalter *et al.* (1985). Such an absence of HRGP mRNA induction was also observed by Dhawale *et al.* (1989) in soybean leaves inoculated with *Pseudomonas syringae* van Hall, whereas HRGP mRNA accumulated in soybean roots infected by *Phytopthora megasperma* Drech. f. sp. *glycinea* T. Kuan & D. C. Erwin (Dhawale *et al.* 1989).

In the three interactions studied, whatever the time course

of the infection progress, a correlation was observed between the accumulation of defense gene transcripts, extensive fungal growth, and the brownish color of the leaf veins. The differences observed after bean infection with races 1 and 2 of *C. lindemuthianum* (race α) and with race 9 (race γ) may be ascribed to some fungal pathogenicity genes, such as hydrolytic enzymes. A pectin lyase has been shown to be produced by *C. lindemuthianum* within spreading lesions (Wijesundera *et al.* 1984). Race pathogenicity seems to vary easily, because it is commonly observed that laboratory cultures of *C. lindemuthianum* repeatedly transferred on agar media will frequently change in pathogenicity (F. Legrand and J. Tailler, laboratoire de Cryptogamie, Université Paris Sud, Orsay, France, personal communication).

The knowledge of the genetic relatedness between the three races used in these studies will allow a better understanding of the mechanisms involved. Are these three races very close genetically or do these three races differ only by some discrete genomic regions? An RFLP and/or a random-amplified polymorphic DNA analysis of the genome of the three races will allow us to answer this question.

A delay in plant defense gene mRNA induction has been observed in compatible interactions when compared to incompatible interactions. This was shown for PAL, CHS (Bell *et al.* 1986), CHI (Mehdy and Lamb 1987), CHT (Hedrick *et al.* 1988), and HRGP (Corbin *et al.* 1987) mRNAs after bean infection with race γ (virulent) and race β (avirulent) of *C. lindemuthianum*. This was also shown for PAL and CHS mRNAs after inoculation of soybean with *P. m.* f. sp. *glycinea* races 1 (avirulent) and 3 (virulent) (Habereder *et al.* 1989) and for chitinase mRNA in bean challenged with virulent (race 3) and avirulent (race 1) isolates of *P. s.* pv. *phaseolicola* (Burkholder) Young *et al.* (Voisey and Slusarenko 1989). In *Arabidopsis thaliana* (L.) Heynh., the BG3 β-1,3-glucanase gene is activated early and only very slightly in an incompatible interaction with an avirulent strain of *P. s.* pv. *tomato* (Okabe) Young *et al.* Thus, the mRNA accumulates much later and to a greater extent in a compatible interaction with a virulent strain of *P. s.* pv. *maculicola* (McCulloch) Young *et al.* (Dong *et al.* 1991). From such studies, the idea that a major determinant of success in a resistance strategy lies in a more rapid defense response has emerged.

In contrast to all these data, in *A. thaliana*, the kinetics of PAL (early and transient) and the BG2 β-1,3-glucanase (late and extended) mRNA induction were the same in incompatible and compatible interactions with *P. s.* pv. *tomato* and *P. s.* pv. *maculicola*, respectively (Dong *et al.* 1991). The kinetics of PAL and 4-coumarate, CoA ligase (4CL), mRNA inductions were also similar in incompatible and compatible interactions of potato with *P. infestans* (Mont.) de Bary races 1 and 4, respectively (Fritzemeier *et al.* 1987).

For all the studies described above, comparisons between incompatible and compatible interactions have been performed with two different races of the pathogen, a virulent race (compatible) or an avirulent race (incompatible). The evidence presented in this report clearly demonstrates that different virulent races were able to induce defense mechanisms with different speed. So, PAL, CHI, and CHT mRNAs were induced 32 hr earlier after infection with race 9 than after infection with races 1 and 2. A delay of about 70 hr was reported for these mRNA inductions when compatible interactions were compared to incompatible ones (Bell *et al.* 1986; Mehdy and Lamb 1987; Hedrick *et al.* 1988). Altogether, these observations suggest that the specific effect of recognition between the avirulence gene of the pathogen and the resistant gene of the cultivar may be confused with the effects exerted by the genetic background of the two races. To avoid this problem, nearly isogenic pathogen races differing by the presence of a single avirulence gene or of plant near-isogenic lines (NILs) differing by the presence of a single resistance gene would be very helpful and powerful model systems. Using such plant NILs, Pautot *et al.* (1991) have reported a differential accumulation of proteinase inhibitors I and II (PI I and II) mRNA in tomato in response to *P. s.* pv. *tomato*. PI II accumulated more rapidly in disease-resistant than in susceptible lines, whereas PI I mRNA was first detected in the compatible interaction. PI I and II genes appeared to be activated by different signal transduction pathways.

Davidson *et al.* (1987) have also used plant NILs with distinct resistance genes to study interactions between barley and *Erysiphe graminis* DC. f. sp. *hordei* Ém. Marchal. The mRNA-specific species of infected leaves have been shown to be induced during the primary penetration processes in both NILs, but four of them are induced in greater amounts in the resistant line.

Thus, the widespread idea that incompatible interactions may be characterized by a faster reaction than compatible interactions cannot be generalized to all systems and to all defense products. To reach an unambiguous insight on the molecular mechanisms underlying genetically controlled race-cultivar specificity, the use of plant-pathogen NILs or of a resistance-avirulence gene disruption methodology will be required.

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