Planta (1989) 177:58–65

Planta

Rapid induction of phenylalanine ammonia-lyase and chalcone synthase mRNAs during fungus infection of soybean (*Glycine max* L.) roots or elicitor treatment of soybean cell cultures at the onset of phytoalexin synthesis

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Abstract. The differential regulation of the activities and amounts of mRNAs for two enzymes involved in isoflavonoid phytoalexin biosynthesis in soybean was studied during the early stages after inoculation of primary roots with zoospores from either race 1 (incompatible, host resistant) or race 3 (compatible, host susceptible) of *Phytophthora megasperma* f.sp. *glycinea*, the causal fungus of root rot disease. In the incompatible interaction, cloned cDNAs were used to demonstrate that the amounts of phenylalanine ammonia-lyase and chalcone synthase mRNAs increased rapidly at the time of penetration of fungal germ tubes into epidermal cell layers (1–2 h after inoculation) concomitant with the onset of phytoalexin accumulation; highest levels were reached after about 7 h. In the compatible interaction, only a slight early enhancement of mRNA levels was found and no further increase occurred until about 9 h after inoculation. The time course for changes in the activity of chalcone synthase mRNA also showed major differences between the incompatible and compatible interaction. The observed kinetics for the stimulation of mRNA expression related to phytoalexin synthesis in soybean roots lends further support to the hypothesis that phytoalexin production is an early defense response in the incompatible plant-fungus interaction. The kinetics for the enhancement of mRNA expression after treatment of soybean cell suspension cultures with a glucan elicitor derived from *P. megasperma* cell walls was similar to that measured during the early stages of the resistant response of soybean roots.

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Abbreviations: cDNA = copy DNA; CHS = chalcone synthase; PAL = phenylalanine ammonia-lyase

Key words: Cell culture (enzyme induction) – Chalcone synthase – Elicitor, fungal – *Glycine* (enzyme induction) – Isoflavonoid phytoalexin – Phenylalanine ammonia-lyase – Phytoalexin synthesis *Phytophthora* – RNA hybridization

Introduction

Higher plants possess a number of inducible defense mechanisms by which they protect themselves against potential pathogens (Bell 1981). One of the best studied is the production of phytoalexins, low-molecular-weight antimicrobial compounds, at the site of attempted infection (Bailey and Mansfield 1982). The phytoalexin response can be induced not only by infection but also by exposure of different plant tissues to biotic or abiotic elicitors (Darvill and Albersheim 1984). In legumes, isoflavonoid-type phytoalexins are of particular importance. They are synthesized by general phenylpropanoid and subsequent specific branch pathways (Ebel 1986).

To investigate the mechanisms underlying the inducible production of isoflavonoid phytoalexins, we are studying two systems. One utilizes the inoculation of soybean (*Glycine max* L.) primary roots with the fungal pathogen *Phytophthora megasperma* f.sp. *glycinea*, the causal agent of root and stem rot, and mimics the natural infection process. The other exploits soybean cell-suspension cultures and a β-glucan elicitor isolated from the fungal cell walls as an experimental system of reduced complexity. These two systems are especially valuable for the analysis of several aspects of the inducible phytoalexin response, including the dynamics of phytoalexin accumulation in relation to the expres-

sion of race- or cultivar-specific resistance, the regulation of enzymes of phytoalexin biosynthesis, and the signal processes involved in the triggering by external stimuli of intracellular responses leading to phytoalexin production (Ebel 1986).

Previous studies have shown that phytoalexin accumulation in infected soybean roots or hypocotyls and in elicitor-treated soybean cell cultures is associated with rapid increases in the activities of at least ten enzymes of the biosynthetic pathway (Ebel 1986). For two enzymes, phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS), the activity increases are correlated with transient increases in their rates of synthesis both in vivo and in vitro (Börner and Grisebach 1982; Hille et al. 1982; Ebel et al. 1984). In preliminary experiments, changes in the amounts of the mRNAs encoding PAL and CHS were investigated (Schmelzer et al. 1984; Esnault et al. 1987). The results were, however, not definite. In their studies, Schmelzer et al. (1984) measured quantitatively changes in the amounts of CHS mRNA, but because only one race of the fungus (race 1) was used, the relationship between expression of disease resistance and increases in CHS mRNA remained unclear. In addition, the levels of PAL mRNA could not be quantitatively determined, possibly because of a low degree of cross-hybridization of the mRNA from soybean with the cDNA from parsley (Schmelzer et al. 1984). In the later studies by Esnault et al. (1987), only qualitative changes in the amounts of mRNA reportedly coding for PAL and CHS were determined. Furthermore, the use of two separate cultivars of soybean, although differing in resistance to *P. megasperma*, makes a direct comparison of changes difficult to relate in terms of host specificity. Nor was it shown whether the copyDNAs (cDNAs) utilized in the studies hybridized to mRNAs of the expected sizes.

Recently, a detailed analysis of a single soybean cultivar showed marked differences in the dynamics and sites of accumulation of glyceollin I, the major phytoalexin of soybean, following infection of roots with zoospores of (i) a virulent (compatible interaction, plant susceptible) and (ii) an avirulent race (incompatible interaction, plant resistant) of *P. megasperma* (Hahn et al. 1985). Using the root-zoospore system, we now report in detail early changes in the amounts of mRNA and in mRNA-linked translational activity for two enyzmes related to glyceollin biosynthesis, PAL and CHS. The speed of the enhancement in the mRNA levels observed during the incompatible root-fungus interaction further strengthens the hypothesis that phytoalexin production is an important early defense response of soybean against *P. megasperma*. We have also compared the kinetics of changes in the respective mRNA levels following root infection or elicitor treatment of cell cultures. We conclude from our results that the timing of the induced increases in the mRNA levels encoding the two enzymes in soybean cell cultures resembles that observed in soybean root tissue during the incompatible interaction.

Materials and methods

Materials. Radiochemicals were purchased from Amersham Buchler (Braunschweig, FRG); restriction endonucleases and RNase A were from Boehringer (Mannheim, FRG); antisera were kindly provided by Dr. K. Hahlbrock (Max Planck Institut für Züchtungsforschung, Köln, FRG) and plasmids pPAL5 and pCHS1 by Dr. C.J. Lamb (The Salk Institute, San Diego, USA). For nick translation we used the kit from GIBCO/BRL (Eggenstein, FRG); for oligo labeling the kit from Pharmacia (Freiburg, FRG) was used.

Fungal cultures. Phytophthora megasperma Drechs f.sp. *glycinea* Kuan and Erwin races 1 and 3 were obtained from Dr. E. Ziegler (RWTH Aachen, FRG) and were grown as described by Ayers et al. (1976). Zoospores were obtained from 6-d-old cultures according to a published method (Eye et al. 1978).

Soybean seedlings and infection. Seeds of soybean (*Glycine max* (L.) Merr. cv. Harosoy 63) were obtained from Dr. R.I. Buzzell (Agriculture Canada, Research Station, Harrow, Canada). Seedlings were grown under aseptic conditions on wet filter paper as described previously (Hahn et al. 1985). The taproot of 1.5-d-old seedlings was inoculated with a suspension of about 10^4 zoospores in 100 µl sterile distilled water by dip-inoculation (Hahn et al. 1985). Control, uninfected seedlings were placed in 100 µl water.

Plant cell cultures and elicitor treatment. Soybean cell-suspension cultures (*Glycine max* L. cv. Harosoy 63) were grown in the dark as described previously (Hille et al. 1982; Ebel et al. 1984) and treated with *P. megasperma* elicitor (80 µg glucose equivalents/ml medium) obtained by partial acid hydrolysis of purified cell walls of the fungus (Sharp et al. 1984), as described earlier (Schmidt 1986).

Tissue extraction and enzyme assays. Excised frozen segments from 18 roots (about 0.6 g) per assay were extracted as described previously (Bonhoff et al. 1986a). Crude cell-free extracts from frozen cell cultures were obtained as described by Ebel et al. (1984). Standard procedures were used to measure the activities of PAL (Zimmermann and Hahlbrock 1975), CHS (Schröder et al. 1979a), and protein content (Bradford 1976).

Isolation of RNA. Total RNA from excised frozen root segments (0.6–1.8 g per assay) or cell cultures (3 g) was isolated by the method of Langridge et al. (1982; see also Ebel et al. 1984). The ethanol-precipitated RNA was washed twice with 3 M sodium acetate, pH 6 (Palmiter 1974), dissolved in water, and again precipitated with ethanol.

Protein synthesis in vitro. RNA was translated in vitro in the presence of [^{35}S]methionine with an mRNA-dependent rabbit reticulocyte lysate (Pelham and Jackson 1976) and incorpora-

tion of radioactivity into total protein was measured as previously described (Ebel et al. 1984). PAL and CHS were immunoprecipitated with specific antisera (Schröder et al. 1979b), combined with adsorption to protein A-Sepharose CL-4B. Proteins from the precipitates were dissolved and subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) (Ebel et al. 1984). Enzyme subunits were located by fluorography (Bonner and Laskey 1974) and incorporation of radioactivity was quantified by scanning fluorographs with a Laser densitometer (LKB, Bromma, Sweden). PAL and CHS mRNA activity is defined as the incorporation of [^{35}S]methionine into the enzyme subunits isolated by gel electrophoresis as a percentage of incorporation into total protein (Ebel et al. 1984).

RNA blot and dot-blot hybridization. Plasmids pPAL5 (Edwards et al. 1985) and pCHS1 (Ryder et al. 1984) were used to transform *Escherichia coli* strain JA221 (Nakamura and Inouye 1982). Following selection of transformants, plasmids were isolated from cleared lysates (Kahn et al. 1979) according to the method of Birnboim (1983) and digested with PstI and EcoRI, respectively (Ryder et al. 1984; Edwards et al. 1985). The cDNA inserts were purified by electrophoresis on 0.8% agarose gels and isolated by electroelution. The PAL cDNA was labeled by oligo labeling (Feinberg and Vogelstein 1983), and CHS cDNA by nick translation with DNA polymerase I and α-[^{32}P]deoxycytidine 5′-triphosphate (dCTP).

For blot hybridization, RNA (10 or 20 μg total RNA) was denatured with formaldehyde, separated by electrophoresis on a 1.2% agarose gel in 40 mM 3-(N-morpholino)propanesulfonic acid buffer, pH 7, containing 10 mM sodium acetate, 1 mM ethylene diaminetetraacetic acid (EDTA), and 2.2 M formaldehyde (Maniatis et al. 1982), and blotted onto nitrocellulose. Blotted RNA was hybridized to ^{32}P-labeled cDNAs for 42 h at 42° C in 45% formamide, 0.8 M NaCl, 45 mM sodium phosphate, pH 7, 4.6 mM EDTA, 0.09% SDS, containing 100 μg denatured herring sperm DNA per ml and 0.2% each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll 400. Hybridized cDNA was located by autoradiography (see below).

Dot blots were obtained according to the procedure of Groner et al. (1982) by applying denatured RNA onto a nitrocellulose filter (presoaked in 10 × SSC; Maniatis et al. 1982) using a hybridot manifold device. Each dot was washed with 150 μl 10 × SSC. The filter was air-dried and baked for 4 h at 80° C and hybridization was carried out as described above. Following autoradiography at −70° C with Cronex-2 film (Du Pont, Newtown, Conn., USA) and intensifying screens for 6–36 h, radioactivity was quantified either directly by scintillation spectrometry of appropriate areas of the nitrocellulose filter or indirectly by eluting the silver grains from autoradiograms and measuring their absorbance at 500 nm (Suissa 1983). For cell-culture samples, relative data were normalized with the darkest band being 100%. For root samples, relative data were normalized with the weakest band (0 h infection). Film darkness or radioactivity was directly proportional to the amount of RNA applied.

Results

Enhancement of the activities of enzymes involved in phytoalexin biosynthesis. The natural infective propagules of *P. megasperma* are zoospores, which frequently infect the soybean root. Dip-inoculation of 1.5-d-old soybean seedling roots with zoospores from either an avirulent (race 1) or virulent (race 3) race of *P. megasperma* resulted in typical incompatible or compatible interactions with the seedlings (Fig. 1). The changes in the activity for one enzyme of isoflavonoid phytoalexin biosynthesis, CHS, which were measured after 8 h of infection, are shown in Table 1. There was a significantly larger increase in activity during the incompatible interaction as compared with the compatible interaction. This result is in agreement with previously observed differences in the kinetics of induction of several enzymes involved in phytoalexin biosynthesis (Bonhoff et al. 1986a, b) and of phytoalexin accumulation (Hahn et al. 1985) following infection of soybean roots with either an avirulent or virulent race of *P. megasperma*. In addition (Table 1), a 10-h treatment of soybean cell-suspension cultures with a β-glucan elicitor from *P. megasperma* resulted in large increases in the activities of PAL and CHS at the onset of phytoalexin accumulation, as reported previously (Hille et al. 1982; Ebel et al. 1984).

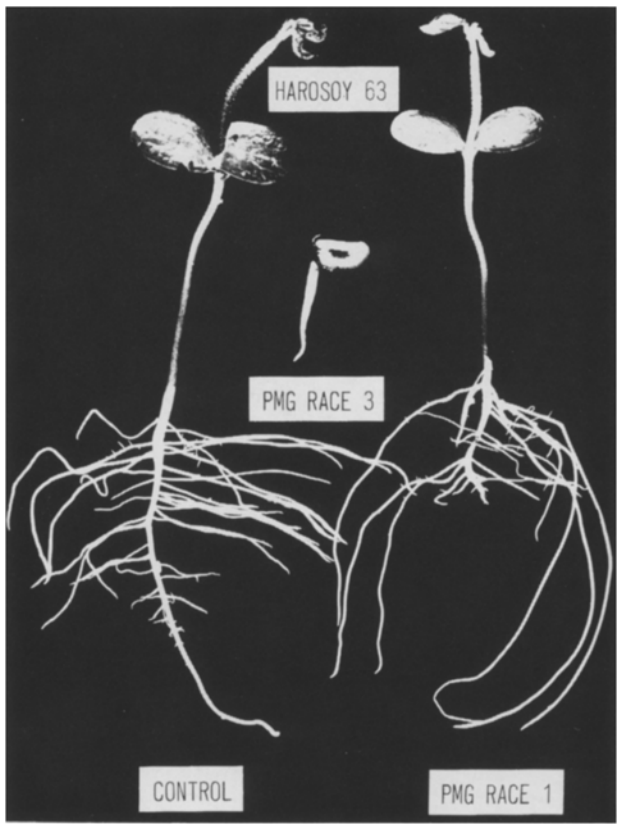

Fig. 1. Soybean seedlings (cv. Harosoy 63) 6 d after inoculation with zoospores of race 1 or race 3 of *P. megasperma* f.sp. *glycinea* (*PMG*). Seedlings (36 h after sowing) were dip-inoculated with zoospores for 2 h, and then planted in sterile vermiculite. Control was treated with sterile water

Table 1. Enhancement of the activity of chalcone synthase following infection of soybean roots with *P. megasperma* and of the activities of phenylalanine ammonia-lyase and of chalcone synthase after elicitor treatment of soybean cell cultures. Means ± SE ($n=4$ for roots; $n=3$ for cell cultures)

Plant tissue	Treatment	h	Enzyme activity (μkat · kg^{-1})	
			Phenylalanine ammonia-lyase	Chalcone synthase
Roots	None	8	–[a]	0.14 (±0.06)
	P. megasperma, race 1	8	–	0.47 (±0.10)
	P. megasperma, race 3	8	–	0.24 (±0.12)
Cell cultures	None	10	5.8 (±1.2)	0.16 (±0.03)
	P. megasperma elicitor	10	82 (±11)	9.6 (±2.4)

[a] Not determined

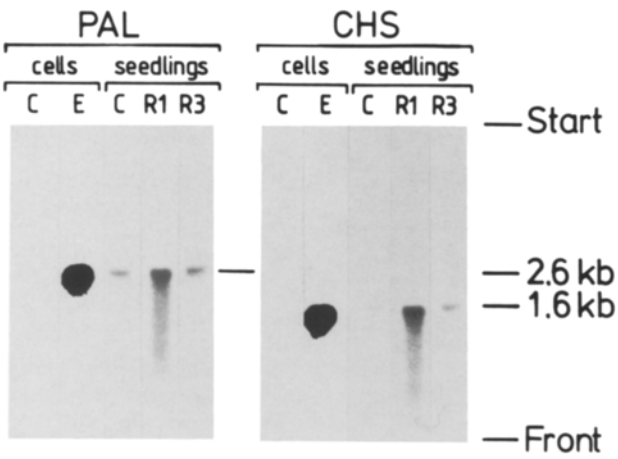

Fig. 2. Blot hybridization of total RNA from soybean cell cultures and roots with ^{32}P-labelled phenylalanine ammonia-lyase (*PAL*) and chalcone synthase (*CHS*) cDNAs after electrophoretic separation of the RNA samples. Total RNA was extracted from either cell cultures or roots which had been treated with β-glucan elicitor or *P. megasperma* zoospores, respectively, for 5 h. Approximate lengths of mRNAs (in kilobases, kb) were estimated using RNA-size markers; *C*, control cell cultures or roots; *E*, elicitor-treated cells; *R1*, roots infected with *P. megasperma* race 1; *R3*, roots infected with race 3

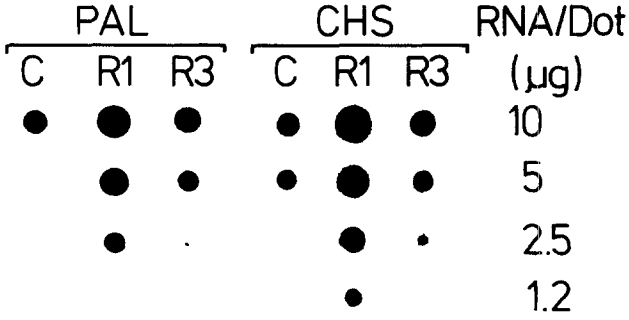

Fig. 3. Dot-blot hybridization of total RNA from soybean roots with ^{32}P-labeled PAL and CHS cDNAs. Serial dilutions of RNA samples from roots inoculated for 5 h were subjected to analysis. *R1*, roots infected with *P. megasperma* race 1; *R3*, roots infected with race 3; *C*, control roots

RNA blot and dot-blot analysis of soybean mRNAs. Heterologous ^{32}P-labeled cDNA probes for bean cell-culture PAL and CHS mRNAs (Ryder et al. 1984; Edwards et al. 1985) were used to detect the respective mRNAs from soybean tissues in RNA-hybridization experiments. In RNA blot hybridizations with electrophoretically separated total RNA from soybean cell-suspension cultures the respective cDNAs hybridized specifically to mRNA species of only one size (Fig. 2). PAL mRNA migrated to a position corresponding to a size of 2.6 kilobases (kb), whereas CHS mRNA displayed a size of 1.6 kb. Similar results were obtained with total RNA fractions from soybean roots (Fig. 2), although some degradation of RNA could be observed in the samples from seedlings that had been infected with *P. megasperma* race 1 for 5 h. The sizes determined for the soybean PAL and CHS mRNAs were very similar to those calculated for the respective bean mRNAs (Ryder et al. 1984; Edwards et al. 1985). Dot-blot hybridizations of RNA were carried out for quantitative assessment of changes of mRNA amounts in response to either infection of soybean roots or elicitor treatment of soybean cell cultures. As an example, both cDNAs hybridized in a quantitative manner with total RNA from soybean roots following various inoculations for 5 h (Fig. 3). Under the conditions used, determination of the levels of PAL transcripts with ^{32}P-cDNA obtained by oligo labeling was superior to that with nick-translated ^{32}P-cDNA. Thus, for quantitative measurements of PAL-mRNA levels, oligo-labeled ^{32}P-cDNA was used, whereas for determination of CHS-mRNA levels nick-translated cDNA was applied.

Induction of mRNA in roots following infection. Changes in PAL- and CHS-mRNA levels were examined in roots of 1.5-d-old soybean seedlings fol-

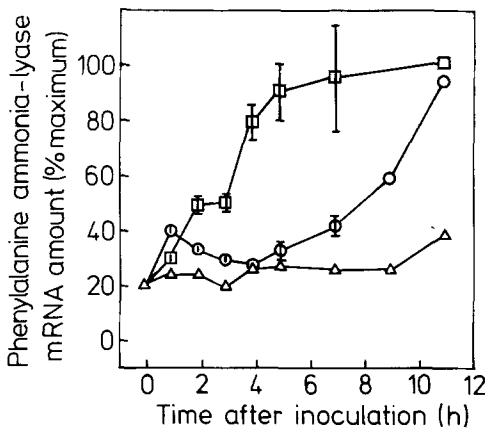

Fig. 4. Changes in the amounts of PAL mRNA in soybean roots after inoculation with zoospores of *P. megasperma* race 1 (□–□) or race 3 (○–○) and in water controls (△–△). Samples were taken from the taproots at the indicated times and RNA levels were determined by dot-blot hybridization in serial dilutions from total RNA of the taproots as indicated in **Fig. 3**. *Bars* represent 2 × SD from three separate experiments ($n=3$). Relative data were normalized with the mRNA amount at 0 h representing 21%

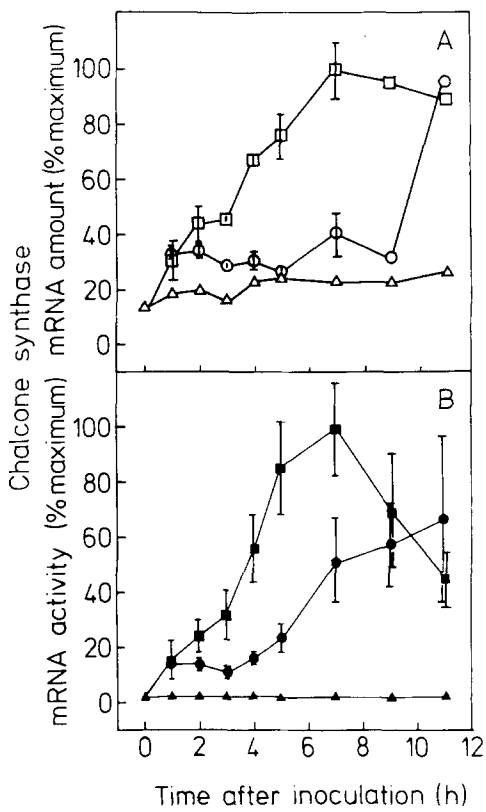

Fig. 5A, B. Time course for CHS-mRNA induction in soybean roots after inoculation with zoospores of *P. megasperma*. Samples were taken at the indicated times after infection with race 1 (□,■) or race 3 (○,●) or mock inoculation with water (△,▲). (**A**) mRNA amount as determined by dot-blot hybridization. *Bars* represent 2 × SD ($n=3$). Relative data were normalized with the mRNA amount at 0 h representing 15%. (**B**) Translational activity in vitro

lowing infection for various periods of time with zoospores from either race 1 or race 3 of *P. megasperma* (Figs. 4, 5). Inoculation with both the avirulent race 1 and the virulent race 3 resulted in the rapid accumulation of PAL (Fig. 4) and CHS mRNAs (Fig. 5) in root tissue which included the sites of infection. Small, but significant increases in mRNA levels for both enzymes were detectable as early as 1 h after inoculation. In the incompatible interaction, the mRNA levels continued to increase rapidly reaching a maximum after about 7 h. In the compatible interaction, no further increase in mRNA amounts occurred until about 9 h after inoculation. In RNA blot hybridization experiments, after separation of total RNA by electrophoresis, the cDNAs for the two enzymes hybridized specifically to only one mRNA band, respectively, over the time period investigated. The time course of changes in CHS-mRNA activity, as measured by in-vitro translation of total RNA in a reticulocyte lysate system, also showed marked differences between the incompatible and compatible interaction (Fig. 5). There were, however, apparent quantitative differences between the patterns for amount and activity of CHS mRNA which cannot be explained at present. Control roots treated with sterile water exhibited low basal PAL- and CHS-mRNA amounts and activities over the period studied.

Induction kinetics of mRNAs in cell cultures following elicitor treatment. The kinetics of induction of PAL and CHS mRNAs were also investigated in soybean cell-suspension cultures following treatment with a β-glucan elicitor obtained by partial hydrolysis of purified cell walls of *P. megasperma*. A detailed examination using cDNAs specific for the bean cell-culture enzymes (Ryder et al. 1984; Edwards et al. 1985) showed (Table 2) that the mRNA levels for both PAL and CHS greatly increased in response to elicitor, the highest levels being about 25- and 70-fold larger as compared with control unelicited cells.

Following elicitor treatment the levels of PAL and CHS mRNA increased after 1–2 h, reached a maximum after 6 h, and then declined (Table 2). Similar overall changes were measured for the respective mRNA activities. In control cell cultures, only low basal mRNA levels and activities for both enzymes were detected throughout the period investigated.

Table 2. Time course for the induction of phenylalanine-ammonia-lyase and chalcone-synthase mRNA in soybean cell cultures following treatment with a β-glucan elicitor derived from *P. megasperma*. Translational activity was determined with a rabbit reticulocyte lysate; mRNA amount was measured by dot-blot hybridization. Means ± SE ($n=3$)

Treatment	h	mRNA activity (%)		mRNA amount (%)	
		Phenylalanine ammonia-lyase	Chalcone synthase	Phenylalanine ammonia-lyase	Chalcone synthase
None	0–10[a]	0	0	4 (±1.8)	1.4 (±0.9)
P. megasperma elicitor	1	0	0.9	4.5 (±1.8)	3.9 (±0.6)
	2	0	9	5 (±0.8)	12 (±1.6)
	3	7	30	26 (±12)	23 (±4)
	4	34	52	34 (±6)	36 (±1)
	5	60	83	81 (±4)	85 (±4)
	6	100	100	100	100
	8	53	80	64 (±3)	72 (±4)
	10	32	76	52 (±9)	78 (±3)

[a] Control samples were taken hourly in parallel with elicitor-treated samples; average values are reported

Discussion

Infection of soybean roots with *Phytophthora megasperma* f.sp. *glycinea* has been utilized for studying several aspects of pathogenesis on susceptible and resistant soybean plants during the development of *Phytophthora* root rot. The naturally infective form of this fungus is the zoospore, and histological investigations, either with soybean cultivars differing in resistance (Beagle-Ristaino and Rissler 1983) or with races of *P. megasperma* differing in virulence (Hahn et al. 1985; J. Golecki, Institut für Biologie II, Universität Freiburg, FRG, personal communication) have been reported to show rapid penetration of root tissues, within 1 to 2 h after inoculation, irrespective of the race-cultivar combination. Biochemical studies have indicated marked changes in root responses associated with these interactions. In the incompatible interaction, isoflavonoid phytoalexins accumulated rapidly and in large amounts during the first 14 h of the infection, whereas in the compatible interaction only comparatively small amounts were detected (Hahn et al. 1985). Thus, it appears likely that, in the resistant response, invading hyphae come into contact with an antimicrobial environment soon after infection (penetration).

We have recently reported (Bonhoff et al. 1986a, b) that during the early stages of infection (2 to 8 h) race- or cultivar-specific differences were observed in the enhanced levels of activity of PAL and CHS, concomitant with other enzymes of isoflavonoid phytoalexin biosynthesis. In the present work, we have detected and measured changes in the respective mRNAs encoding the two former enzymes, using heterologous cDNA probes complementary to bean (*Phaseolus vulgaris*) PAL and CHS mRNAs because probes from soybean had not yet been developed. The bean cDNAs hybridized specifically and quantitatively to mRNA species from soybean roots and cell cultures which were of the sizes (2.6 and 1.6 kb) expected from the work with bean (Ryder et al. 1984; Edwards et al. 1985). We therefore concluded that cross-hybridization of the heterologous cDNAs was sufficient for reliable quantification of soybean PAL and CHS mRNA. The hybridization method thus allowed a detailed analysis of the kinetics of production of the respective mRNAs.

Following infection of soybean roots with zoospores of *P. megasperma*, a rapid induction was observed in the amounts and activities of the mRNAs for PAL and CHS; these changes preceded increases in the activity levels of the corresponding enzymes (Bonhoff et al. 1986a, b). Furthermore, after a slight enhancement in the first 2 h following inoculation, the kinetics of increases in the mRNA levels in the incompatible and compatible interaction were, at later periods, strikingly different. However, the differential enhancement of the mRNA levels in the two types of interaction exactly reflects the timing of increases in the levels of activity of biosynthetic enzymes and phytoalexin accumulation as observed earlier. It can, therefore, be assumed that regulation of mRNA amounts is an important early component of the overall control of the phytoalexin defense respone in soybean roots. A similar conclusion has been reached in other plant-parasite systems (for review, see Lamb et al. 1986; Scheel et al. 1986). The timing of mRNA induction related to phytoalexin formation in the soybean-*P. megasperma* interaction

is, however, very rapid when compared with that of one of the other systems studied in some detail. In an incompatible *Phaseolus vulgaris-Colletotrichum lindemuthianum* interaction, about 40 h elapsed before enhanced amounts of PAL and CHS mRNAs were measured (Lamb et al. 1986). The rapidity of mRNA induction in soybean might imply that at least some of the components involved in the perception and transduction of the signal produced by the invading fungus in the soybean roots might be available within a short period after inoculation.

The earliest measurable increases in the amounts of mRNAs encoding enzymes of phytoalexin biosynthesis have been observed at the time of fungal germ-tube penetration of the rhizodermis (Beagle-Ristaino and Rissler 1983). It is possible that the epidermal cell layers are those responsible for the early and strong mRNA induction, as has been concluded for phytoalexin accumulation in the incompatible interaction (Hahn et al. 1985). Further experiments at the cellular level are required to establish the spatial relationship between mRNA induction and phytoalexin accumulation.

Another interesting observation was that the kinetics of mRNA induction in the incompatible root-fungus interaction was very similar to that observed after elicitor treatment of soybean cell-suspension cultures. One major difference between the two systems was that the induced specific mRNA levels were about ten-times higher in cell cultures than in roots. The difference in the level of induction may, at least in part, be a consequence of the fact that in the cell culture all cells are exposed at the same time to the elicitor stimulus, whereas in the incompatible plant root-fungus interaction the phytoalexin defense response is highly localized (Hahn et al. 1985) and a major portion of the root tissue subjected to analysis has not yet been challenged by the fungus. Similar large differences between the two systems have also been observed for the induced activity levels of PAL and CHS (Table 1 and Bonhoff et al. 1986a). For soybean roots it has thus now been established that not only phytoalexin accumulation itself but also the induction of phytoalexin biosynthetic enzymes and their mRNAs reflect the known differences in the physiological interaction between different races of *P. megasperma* and the soybean plant.

This work was supported by the Deutsche Forschungsgemeinschaft, Fonds der Chemischen Industrie, and BASF (Ludwigshafen, FRG). We thank Dr. K. Hahlbrock (Köln) for the provision of antisera, Dr. C.J. Lamb (San Diego, USA) for plasmids, Dr. R.I. Buzzell (Harrow, Canada) for soybean seeds, and Dr. D.R. Biggs for helpful comments on the manuscript.

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Received 2 April; accepted 19 August 1988