Race cultivar-specific differences in callose deposition in soybean roots following infection with Phytophthora megasperma f.sp. glycinea

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Abstract. Primary roots of soybean (Glycine max (L.), Merrill, cv. Harosoy 63) seedlings were inoculated with zoospores from either race 1 (incompatible, host resistant) or race 3 (compatible, host susceptible) of Phytophthora megasperma f.sp. glycinea and total callose was determined at various times after inoculation. From 4 h onward, total callose was significantly higher in roots showing the resistant rather than the susceptible response. Local callose deposition in relation to location of fungal hyphae was determined in microtome sections by its specific fluorescence with sirofluor and was quantified on paper prints with an image-analysis system. Callose deposition, which occurs adjacent to hyphae, was found soon after inoculation (2, 3 and 4 h post inoculation) only in roots displaying the resistant response, and was also higher at 5 and 6 h after inoculation in these resistant roots than in susceptible roots. Early callose deposition in the incompatible root-fungus reaction could be a factor in resistance of soybean against P. megasperma.

Key words: Glycine (callose and Phytophthora infection) – Phytophthora – Callose – Root (infection).

Introduction

For biochemical investigations on Phytophthora rot of soybean we have used an inoculation system which mimics as closely as possible the natural infection process (Hahn et al. 1985). Young unwounded soybean seedlings are inoculated at the tap root with zoospores of an incompatible race (race 1, host resistant) or a compatible race (race 3, host susceptible) of the fungus Phytophthora megasperma f.sp. glycinea. In this system we have investigated race cultivar-specific accumulation of glyceollin, the phytoalexin of soybean (Hahn et al. 1985) and race cultivar-specific induction of enzymes involved in glyceollin biosynthesis during the initial period after infection (Bonhoff et al. 1986a, b)

The importance of glyceollin in the defense reaction of soybean has been shown in experiments where the synthesis of this phytoalexin was inhibited by L-2-aminooxy-3-phenylpropionic acid (Moesta and Grisebach 1982). The results of these studies have recently been corroborated and extended (Waldmüller and Grisebach 1987) by using a new inhibitor for phenylalanine ammonia-lyase, R-(1-amino-2-phenylethyl)phosphonic acid (Laber et al. 1986). While these studies and data on glyceollin accumulation (Hahn et al. 1985) support the hypothesis that production of glyceollin is an important early defense of soybean roots to infection by P. megasperma, the phytoalexin may not be solely responsible for inhibition of fungal growth in the resistant response (Hahn et al. 1985). We have therefore extended our studies on other race cultivar-specific responses.

In soybean cell-suspension cultures, chitosan (fragments of deacetylated chitin) elicits not only the accumulation of glyceollin but also a rapid deposition of callose in a Ca$^{2+}$-dependent reaction (Köhle et al. 1983, 1985). Extensive investigations have shown a Ca$^{2+}$ dependency of the plasma-membrane-located 1, 3-β-glucan synthase (reviewed in Kauss 1987). We therefore investigated whether callose deposition occurring in soybean roots after infection with P. megasperma is a race-specific response.

Materials and methods

Chemicals. Aniline blue was obtained from Merck (Darmstadt, FRG), sirofluor from Biosupplies (Melbourne, Australia), LR White from London Resin Co. (Basingstoke, U.K.), and lami-
A calibration curve was obtained with laminarin.

Procedures were carried out as described by Köhle et al. (1985). Zoospores were disintegrated in a glass homogenizer (Potter-Elvehjem, Crawfordsville, Indiana, USA) and processed on glass slides. For detection of hyphae the sections were stained with methylene blue/azur II (Richardson et al. 1960). Other sections were treated for 30 min with aniline blue (Köhle et al. 1985). The results are shown in Fig. 1. The callose content in roots showing the resistant response was at 4 h and up to 8 h pi significantly higher than in the susceptible roots. In the latter case, total callose was not very different from that in uninfected roots. At 12 h pi the total callose content in both root-fungus interactions was about equal (data not shown). The results were verified in two further experiments. It was therefore of interest to determine temporal callose deposition in relation to localization of hyphae as shown in microtome sections of infected roots.

Determination of local callose deposition in relation to location of hyphae. For histochemical detection of callose in microtome sections of soybean roots it was of advantage to use sirofluor, a chemically defined fluorochrome isolated as by-product from aniline blue (Stone et al. 1984). Sirofluor gives a brilliant yellow fluorescence with (1→3)-β-D-glucans with very low background fluorescence.

Figures 2 and 3 show callose deposition in a root section 3 h after inoculation with race 1 of P. megasperma. At this time after inoculation with race 3 no callose deposition was visible. Callose deposition was only found at cell walls which were in contact with fungal hyphae. This is in agreement with earlier observations that "papillium-like material" is formed at penetration zones of haustoria from P. megasperma in soybean roots (Slusher et al. 1974).

To verify the chemical nature of the deposition, parallel sections were treated for 1.5 h at 25°C with laminarinase (endo-1,3-β-D-glucanase). After this treatment, fluorescence with sirofluor was

Soybean seedlings. Seeds of soybean (Glycine max (L.) Merrvill cv. Harosoy 63) were obtained from R.I. Buzzell, Harrow, Ontario, Canada. Seedlings were grown under aseptic conditions on wet filter paper as described previously (Hahn et al. 1985).

Fungal culture. Phytophthora megasperma Drechs f.sp. glycinea Kuan and Erwin races 1 and 3 were grown as described by Ayers et al. (1976). Zoospores were obtained from 6-d-old cultures according to Eye et al. (1978).

Root-inoculation procedure. The tap root of 2-d-old seedlings was inoculated with a suspension of about 10⁴ zoospores by dipinoculation as described by Hahn et al. (1985). Controls were placed in 100 μl sterile distilled water.

Determination of total callose. Roots were excised 17 mm from the root tip. Three segments (about 85 mg fresh weight) were used for analysis. To remove autofluorescent material the root segments were soaked for 0.5 h in 2 ml of ethanol. The segments were disintegrated in a glass homogenizer (Potter-Elvehjem, Zurich, Switzerland) in 2.25 ml of 1 N NaOH. The following procedures were carried out as described by Köhle et al. (1985). The fluorescence of callose with aniline blue was determined in a model LS-3 B fluorescence spectrometer (excitation 400 nm, emission 480 nm; Perkin-Elmer, Oberlingen, FRG). A calibration curve was obtained with laminarin.

Determination of callose in microtome section. Excised roots were fixed with 2% formaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 12 h. After fixation, the lower 7 mm of the roots were discarded. The next 6 mm were rinsed in 0.1 M phosphate-buffered saline (PBS), dehydrated by increasing concentrations of ethanol and embedded in LR White according to published methods (Newman et al. 1983). After polymerization the roots were sectioned semithin (1 μm) with an ultramicrotome (Nova; LKB, Bromma, Sweden) for light-microscopical examination. Sections were prepared on glass slides. For detection of hyphae the sections were stained with methyleneblue/azure II (Richardson et al. 1960). For detection of callose the sections were treated for 30 min at 20°C with 20 μl of a solution of sirofluor (0.03 mg.ml⁻¹ in distilled water). Subsequently, the sections were rinsed with water, dried, and the fluorescence detected with a fluorescence microscope (Universal; Zeiss, Oberkochen, FRG) as described by Stone et al. (1984). Lignin was tested on native root sections with phloroglucinol-HCl (Jensen 1962).

Morphometric measurements. The morphometric measurements were made on paper prints at a final magnification of 580- or 1800-fold on an image-analysis system (ASM; Leitz, Wetzlar, FRG) as described by Golecki et al. (1980).

Results

Changes in total callose content in soybean roots post inoculation (pi). The primary roots of 2-d-old soybean (cv. Harosoy 63) seedlings were inoculated with a zoospore suspension of either race 1 (incompatible) or race 3 (compatible) of P. megasperma for various lengths of time. Total callose was determined in the excised root segments with aniline blue (Köhle et al. 1985). The results are shown in Fig. 1. The callose content in roots showing the resistant response was at 4 h and up to 8 h pi significantly higher than in the susceptible roots. In the latter case, total callose was not very different from that in uninfected roots. At 12 h pi the total callose content in both root-fungus interactions was about equal (data not shown). The results were verified in two further experiments. It was therefore of interest to determine temporal callose deposition in relation to localization of hyphae as shown in microtome sections of infected roots.

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