INDUCED RESISTANCE IN *ARABIDOPSIS* AND RADISH: INVOLVEMENT OF PR PROTEINS

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ABSTRACT

This paper demonstrates that *Pseudomonas fluorescens* strain WCS417 and salicylic acid can induce systemic resistance in radish and *Arabidopsis thaliana* against *Fusarium oxysporum*. In neither of the two plant species this induction is associated with induction of PRs. This indicates that PR-accumulation is not a prerequisite for induction of systemic resistance.

INTRODUCTION

In 1961 Ross introduced the term systemic acquired resistance (SAR) to describe the phenomenon that tobacco can be protected against a range of viruses by prior inoculation with TMV. Since then, it is generally assumed that the resistance inducing pathogen must cause necrosis in order to be effective, that is effective against various types of pathogens and that SAR is associated with induction of pathogenesis-related (PR) proteins.

More recently it was demonstrated that also salicylic acid (SA; White, 1979) and non-pathogenic *Pseudomonas fluorescens* (Alström, 1991; Wei *et al.*, 1991; Van Peer *et al.*, 1991) can induce systemic resistance. These inducers do not cause any necrosis. However, there seems to be a high similarity between pathogen- and SA-induced resistance since both TMV- and SA-induced resistance in tobacco appear to involve expression of so-called SAR-genes, among which PRs-1 through -5 (Ward *et al.*, 1991). To our knowledge, no research has been done yet on induction of gene-expression in relation to induced resistance mediated by non-pathogenic *P. fluorescens*.

Our research has been focused on induction of resistance by *P. fluorescens* strain WCS417 against fusarium wilt. This strain induces resistance against *Fusarium oxysporum* in carnation (Van Peer *et al.*, 1991). This paper reports on induction of resistance by the same strain in radish and *Arabidopsis thaliana*. We investigated the nature of induced resistance, with emphasis on the involvement of PR proteins. We compared strain WCS417 as an inducer with SA.

MATERIAL AND METHODS

After germination, radish (*Raphanus sativus* L. cv. Saxa*Nova) and *Arabidopsis* (*A. thaliana* ecotype Columbia) plants were transferred to a rock wool system allowing for spatial separation of an induction treatment and pathogen-inoculation. Subsequently, the induction treatment was applied to the lower part of the roots as

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a talcum suspension, consisting of a mixture of talcum and 10 mM $MgSO_4$ (control), a suspension of strain WCS417 (10^9 cells/mL 10 mM $MgSO_4$) or 2 mM SA. Two days later pathogen inoculation was carried out by placing peat, incubated with spores of *Fusarium oxysporum* f.sp. *raphani* (10^5 cfu/g for radish, 10^6 cfu/g for *Arabidopsis*), on the upper part of the root. For determination of the disease incidence 8 replicates of 6 plants were used for radish, and 20 plants for *Arabidopsis*.

The intercellular fluid (ICF) of radish leaves was collected by vacuum infiltration with a phosphate buffer followed by centrifugation. Leaf and root proteins were isolated by extracting frozen material with a citrate/phosphate buffer (pH 3), centrifugation and subsequent dialysis of the supernatant against ultra pure water. The samples were electrophoresed on a SDS-polyacrylamide (15%) gel and electroblotted onto a nitrocellulose membrane. Antisera against tobacco PR-1a, b and c, PR-2a and b, PR-3 (class I and II) and PR-5 and tomato PR-4 were used to detect homologs in radish. Antigens were visualised after incubation with goat anti-rabbit IgG conjugated to alkaline phosphatase and with BCIP/nitro BT, respectively.

Total RNA was isolated from frozen *Arabidopsis*-leaves using a guanidine HCl extraction method. Total RNA (15 μg) was electrophoresed on denaturing formaldehyde-agarose gels and blotted onto positively charged nylon membranes by capillary transfer. Northern blots were hybridized with a [α-^{32}P]dATP-labelled *A. thaliana* 1,3-ß-glucanase probe (BG2; kindly provided by Dr F.M. Ausubel), derived from a 2 kb *Hin*dIII-insert from plasmid A-2237. After washing in 2 x SSC, 1% SDS they were exposed to a Kodak X-OMAT AR film.

RESULTS

Both radish and *Arabidopsis* showed clear fusarium-wilt symptoms about two weeks after inoculation. In radish, symptoms varied from a mere browning of the vascular root tissue to death of the plant. In *Arabidopsis*, leaves became chlorotic, starting from the veins. Disease symptoms were significantly reduced by prior treatment with strain WCS417 and SA. Disease reduction was manifested differently in the two species: in radish (Table 1) the percentage of diseased plants was reduced, but once infection had occurred, no differences in disease severity were evident. On the contrary, in *Arabidopsis* (Fig. 1) all plants became diseased but the rate at which symptoms developed was significantly reduced.

In neither of the two plant species, any treatment was accompanied by an accumulation of PRs. In radish (Table 2) PR-2 and PR-3 were constitutively present in leaf-ICF and roots, respectively. No increase was detectable upon induction and/or inoculation. In *Arabidopsis* (Table 3) PR-2 mRNA accumulated in the control treatment 24 days post inoculation, probably due to ageing. Plants that were only bacterized showed the same accumulation pattern. Plants treated with both

Table 1. Disease incidence among radish plants, 20 days after inoculation with *F. oxysporum*. Means with the same letter are not significantly different.

Induction treatment	% diseased plants	
	expt 1	expt 2
control	69 [a]	52 [A]
strain WCS417r	50 [b]	12 [B]
2 mM salicylic acid	39 [b]	25 [B]

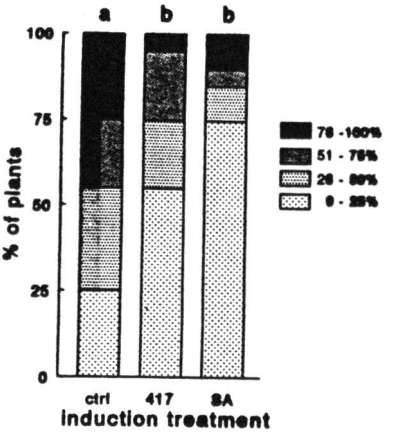

Figure 1. Distribution of *Arabidopsis* plants, 2 weeks after inoculation, among 4 classes of disease severity, depending on the % of leaves showing disease symptoms. Treatments with the same letter are not significantly different.

WCS417 and *F. oxysporum* showed a later accumulation of PR-2 transcripts than plants treated with *F. oxysporum* only, due to retardation of the disease development.

DISCUSSION

These results demonstrate that strain WCS417 is active in inducing resistance against *F. oxysporum* in radish and

Table 2. Occurrence of PRs in radish plants (schematized results of western blot analyses). Samples were collected 4 days after the inoculation treatment.

Inoculation treatment			control			*F. oxysporum*		
Induction treatment			ctrl	417	SA	ctrl	417	SA
PR-1	all samples		-	-	-	-	-	-
PR-2	leaf:	total	-	-	-	-	-	-
		ICF	+	+	+	+	+	+
	root:	upper*	-	-	-	-	-	-
		lower**	-	-	-	-	-	-
PR-3	leaf:	total	-	-	-	-	-	-
		ICF	-	-	-	-	-	-
	root:	upper*	+	+	+	+	+	+
		lower**	+	+	+	+	+	+
PR-4	all samples		-	-	-	-	-	-
PR-5	all samples		-	-	-	-	-	-

* part of the root system which was (mock) inoculated with *F. oxysporum*
** part of the root system to which the induction treatment was applied

Arabidopsis. However, the nature of the induced resistance is different between the two plant species. In radish, resistance is operative during the first stages of fungal root-penetration only, while in *Arabidopsis* resistance seems to be expressed during colonization of the plant by the fungus.

Induction of resistance is not accompanied by specific accumulation of any of the PRs-1 through -5 in radish or PR-2 in *Arabidopsis*. Previously, Uknes *et al.* (1992) reported that SA-induced resistance in *Arabidopsis* is accompanied by expression of PR-1, -2 and -5 genes. The absence of this PR accumulation in our system may be related to the use of the rock wool system.

Table 3. Accumulation PR-2 mRNA in *Arabidopsis* in response to induction by strain WCS417 and/or inoculation with *F. oxysporum* at different days post inoculation (schematized results of northern blot analysis).

Inoculation treatment	control		*F.oxysporum*	
Induction treatment	ctrl	417	ctrl	417
14 dpi	-	-	-	-
20 dpi	-	-	+	-
24 dpi	+	+	+	+

Our results clearly demonstrate that induction of PRs is not a prerequisite for the expression of *Pseudomonas*- and SA-induced resistance against *F. oxysporum* in radish and *Arabidopsis*.

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