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Salicylic acid induced insensitivity to culture filtrate of *Fusarium oxysporum* f.sp. *zingiberi* in the calli of *Zingiber officinale* Roscoe

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Abstract

Salicylic acid (SA) was used to induce insensitivity in the callus cultures of *Zingiber officinale* against culture filtrate (CF) of *Fusarium oxysporum* f.sp. *zingiberi*. The treatment of callus cultures with SA (10^4 μM) prior to selection with CF of the pathogen-increased callus survival. Exogenous application of SA resulted in increased activity of peroxidase and β-1,3-glucanase enzymes in the callus cultures. No increase in the activity of phenylalanine ammonia lyase was obtained. Two new protein bands of ~97 and 38 kDa molecular weights were obtained by SDS-PAGE analysis of soluble proteins extracted from SA-treated calli. The PR-1 monoclonal antibody used for immunodetection of induced proteins cross-reacted with the 38 kDa protein band. *In vitro* antifungal activity of protein extract of calli treated with SA tested against the spores of *F. oxysporum* f.sp. *zingiberi* showed significant reduction in spore germination and germ tube elongation. It is concluded that in ginger, SA may result in the induction of resistance to *F. oxysporum* f.sp. *zingiberi* by inducing increased activity of peroxidase, β-1,3-glucanase and antifungal PR-proteins.

Abbreviations: BA – benzyladenine; CF – culture filtrate; 2,4-D – 2,4-dichlorophenoxyacetic acid; McAb – monoclonal antibody; MS – Murashige and Skoog, 1962; PAL – phenylalanine ammonia lyase; PAGE – polyacrylamide gel electrophoresis; SA – salicylic acid; SAR – systemic acquired resistance; SDS – sodium dodecyl sulphate; TCA – trichloroacetic acid.

Introduction

Fusarium oxysporum f.sp. *zingiberi* Trujillo, causing yellows, is a serious pathogen of ginger in Himachal Pradesh, India. Being rhizome and soil-borne in nature, chemical control of the disease is neither effective nor economically viable. Resistance to this pathogen in any of the ginger cultivars and germplasm is not available. Hence, induction of resistance may be an alternative for the management of ginger yellows. Induction of resistance using salicylic acid (SA) has been reported in a number of host-virus systems (Klessigs et al., 1993; Malamy et al., 1992), as well as in tobacco – *Erwinia carotovora* sub sp. *carotovora* (Palva et al., 1994), rice – *Magnaporthe grisea* (Cai et al., 1996) and cacao – *Phytophthora palmivora* (Okey and Sreenivasan, 1996) systems. Induction of resistance using SA is often accompanied by the formation of pathogenesis-related (PR) proteins first described by Van Loon and Van Kammen (1970) as components of hypersensitive response in the leaves of tobacco plants exposed to TMV. PR-proteins now include all plant proteins that are induced in pathological or related situations (Van Loon et al., 1987) They have been grouped into different PR-protein families based on their primary structure, serological relatedness and enzymatic and biochemical activities (Linthorst, 1991; Van Loon et al., 1993;

Van Loon, 2000). Antifungal activity of PR-1 proteins has also been reported (Niderman et al., 1995). SA can thus act as an effective exogenous inducer of resistance and also of PR-proteins in some host-pathogen systems. The culture filtrate (CF) of *F. oxysporum* f.sp. *zingiberi* produces yellow symptoms in shoots similar to those of pathogen and has been used as *in vitro* selection agent (Prachi et al., 2000). There is no report on the use of SA for the induction of resistance to *F. oxysporum* f.sp. *zingiberi* in ginger or induction of insensitivity of callus cultures to CF of the pathogen. These studies were, therefore conducted to determine the effect of SA induced insensitivity of ginger calli to CF of *F. oxysporum* f.sp. *zingiberi* and for selection of insensitive calli. Biochemical analysis of resistance responses as well as induction of PR-proteins in the SA-treated calli and their antifungal activity against *F. oxysporum* f.sp. *zingiberi* are also described.

Materials and methods

Production of CF

Young cultures (6–8 mm pieces) of *F. oxysporum* f.sp. *zingiberi* were placed in 100 ml of Czapek Dox broth (CDB) (2.0 g $NaNO_3$, 1.0 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4$, 30 g sucrose and 1000 ml distilled water) in 250 ml conical flasks for the preparation of CF. Partial purification of CF was done by the ethyl acetate method of Jin et al. (1996). The aqueous and ethyl acetate phases of CF were separately incorporated into the callus multiplication medium (MS basal medium + 1 mgl^{-1} BA + 0.5 mgl^{-1} 2,4-D). The CF (4, 8 and 12 ml each of aqueous and ethyl acetate phases) was added to 25 ml of callus multiplication medium (Jin et al., 1996). Similar quantities of ethyl acetate and CDB separately added to the callus multiplication medium served as controls. A 20 ml aliquot of each sterilized toxic medium was aseptically poured into sterilized Petri plates (9 cm dia). Calli were placed on these media under aseptic conditions and kept in the dark at 25 ± 1 °C. The phytotoxicity of CF was recorded as plus (calli browning) or minus (no calli browning). The experiments were replicated three times.

Establishment of callus cultures

Callus cultures were established from immature leaf explants of ginger cultivar Dadasiba (Babu et al., 1992). The explants (0.25–0.5 cm) were inoculated on MS basal medium (Murashige and Skoog, 1962) containing 2, 3, 4 and 5 mgl^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) for callus induction in tissue culture tubes (25 × 150 mm, Borosil). These cultures were incubated in the dark at 25 ± 1 °C. After one month, calli were subcultured on MS basal medium containing 2,4-D (0.5 mgl^{-1}) and benzyladenine (BA) (1 mgl^{-1}). The calli were propagated and maintained by repeated subculturing on fresh batches of the same medium.

Treatment of calli with SA

SA was incorporated into callus multiplication medium (MS basal + 1.0 mgl^{-1} BA + 0.5 mgl^{-1} 2,4-D) to get 10, 100, 1000 and 10 000 µM final concentrations. The pH of the medium was adjusted to 5.8 before autoclaving. Healthy, proliferating callus cultures were placed on this medium and incubated for 0, 4, 8, 12 and 24 h at 25 ± 1 °C and 16 h/8 h light and dark period cycles at a light intensity of 140–145 µE m^{-2} s^{-1}. The calli treated with SA for different concentrations and durations were placed on callus multiplication medium supplemented with 0, 4, 8 and 12 ml of partially purified CF of *F. oxysporum* f.sp. *zingiberi*. Observations on the numbers of healthy/dead calli and changes in callus colour were recorded on a 1–5 scale of browning of calli (Jin et al., 1996). Ten callus pieces were placed in each Petri plate with three replications of each treatment.

Determination of enzyme activities in SA-treated calli

The activity of peroxidase and phenylalanine ammonia lyase (PAL) enzymes was determined (Sadasivam and Manickam, 1992) and β-1,3-glucanase activity was measured by the method of Abeles and Forrence (1970).

Extraction of total soluble proteins

Proteins were extracted by grinding 1.0 g of SA-treated callus in 1 ml extraction buffer (250 mM sodium acetate, pH 5.2) with a pre-cooled pestle and mortar (Wubben et al., 1993). The homogenate was centrifuged at 14 000*g* for 10 min at 4 °C in a cooling centrifuge (Beckman, Avanti^{TM30}, F3602 rotor). The supernatant was removed, placed in sterilized tubes.

Before electrophoresis, protein concentrations were standardized by precipitation with 10%

trichloroacetic acid (TCA). For this 10 µl of TCA (40%) was added in 30 µl of protein extract. The samples were incubated at −20 °C for 30 min followed by spinning at 12 000*g* for 10 min in a cooling centrifuge (Beckman, Avanti^{TM30}). Supernatant was decanted, methanol (80%) was added to the pellet and stored at −20 °C for 2 h. Methanol was removed after centrifugation at 12 000*g* for 10 min and the protein pellet was dried. The protein pellet was dissolved in sample buffer (0.0625 M Tris–HCl, pH 6.8, 2% sodium dodecyl sulphate (SDS), 5% 2-mercaptoethanol, 10% sucrose and 0.002% bromophenol blue) to get 2 µg µl^{-1} protein concentration in each sample. The samples were heated in a boiling water bath for 10 min before use in electrophoresis.

SDS-polyacrylamide gel electrophoresis

Separation of total soluble proteins by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 10% slab gels (30 : 0.8 acrylamide : bisacrylamide using a discontinuous buffer system (Laemmli, 1970).

A 40 µl aliquot of each sample (2 µg protein µl^{-1}) was loaded in each well. Electrophoresis was performed in a mini electrophoresis apparatus (Bangalore Genei Pvt. Ltd., India) at 200 V for 90 min. The gels were stained and fixed in a staining solution consisting of methanol (40%), acetic acid (10%) and Coomassie Blue G-250 (0.1%) for 60 min and kept in a destaining solution (10% methanol and 7.5% acetic acid) for 12 h. Molecular weight was estimated by co-electrophoresis of marker proteins (Protein Molecular Weight Marker for SDS-PAGE, Bangalore Genei Pvt. Ltd., India). The marker proteins consisted of a mixture of proteins with different molecular weights, i.e. phosphorylase b (97 400 kDa), bovine serum albumin (69 000 kDa), ovalbumin (43 000 kDa), carbonic anhydrase (29 000 kDa) and lysozyme (14 300 kDa).

Western blotting and immunodetection

After electrophoresis of total soluble proteins of SA-treated calli, Western blotting and immunodetection was performed with monoclonal antibody (McAb) of PR-1 protein of tobacco (obtained from Dr. D.F. Klessig of Waksman Institute, Rutgers, New Jersey, USA) (Hoffland et al., 1996) and by using a Blot Development kit (Bangalore Genei Pvt. Ltd., India).

Determination of in vitro *antifungal activity of PR-proteins*

Total soluble proteins were extracted from SA (10^4 µM) treated calli of ginger in 250 mM sodium acetate buffer (pH 5.2) (Wubben et al., 1993). A spore suspension of *F. oxysporum* f.sp. *zingiberi* containing 5×10^6 spores per ml was used for an *in vitro* test of antifungal activity of PR-proteins. Spore suspension (25 µl) was placed on a cavity glass slide and mixed with 25 µl of test solution containing 125 µg protein. Three replications were used for each treatment. The slides were incubated in a moist chamber at 28 °C for 8 h and observations were recorded as percentage spore germination and germ tube length. The data were based on counting of 100 spores per replication. Sterilized distilled water and protein extraction buffer were included as two separate control treatments.

Protein concentration was determined using BSA as a standard (Bradford, 1976). Data were statistically analyzed and least significant differences (LSD; $P \leq 0.05$) calculated (Gomez and Gomez, 1984).

Results

Induction of insensitivity with SA

The effect of CF of *F. oxysporum* f.sp. *zingiberi* on the calli pre-treated with SA for 24 h was studied and results are given in Table 1. No concentration of SA supplemented in the culture medium was phytotoxic to calli, since the rating for browning of calli was same in the treatments of SA (Table 1). There was an increase in callus survival with the increase in concentration of SA, on a medium containing 4 ml of CF in 25 ml of medium. Callus survival was maximum in calli treated with the highest concentration of SA prior to inoculation on toxic medium. When the concentration of CF was increased to 8 ml in 25 ml of the medium, only 3.3% of calli survived without SA treatment. A reverse trend was obtained for the rating for browning of calli. There was a decrease in the ratings for brown calli with increase in concentration of SA. At the highest concentration of CF, only calli treated with 10^4 µM SA survived (Table 1).

Since the insensitivity of callus cultures was increased after the treatment of calli with 10^4 µM SA for 48 h even at the highest concentration of CF, the next test was to determine the effect of different durations

Table 1. Effect of pre-treatment with different concentrations of SA on survival of calli of ginger cv. Dadasiba exposed to CF of *F. oxysporum* f.sp. *zingiberi*[a]

SA (μM)	CF (ml)[b]							
	0		4		8		12	
	Per cent survival	Calli brown rating[c]	Per cent survival	Calli brown rating	Per cent survival	Calli brown rating	Per cent survival	Calli brown rating
0	100 (89)	1	53 (47)	2.9	3 (6.8)	4	0 (0.9)	4
10^2	100 (89)	1	80 (6 4)	2.1	6.7 (9.5)	3.5	0 (1)	4.5
10^3	100 (89)	1	80 (68)	2	40 (38.9)	3	0 (0.9)	4.5
10^4	100 (89)	1.3	87 (72)	1.9	83 (66)	2.2	83 (66)	2.2
LSD ($P \leq 0.05$)	Conc. of SA	CF concentration						
Per cent survival	7.1	7.1						
Calli brown rating	0.3	0.3						

[a]Average of three replications with 10 calli in each replication.
[b]CF = Culture filtrate; added in 25 ml (v/v) callus culture medium.
[c]Calli brown rating; 1 = no browning, 2 = callus surface slightly brown, 3 = whole tissue brown, 4 = deep brown with restricted growth, 5 = deeply brown with no growth.
SA = Salicylic acid; values in parentheses are angular transformations; 0 = control.

Table 2. Effect of pre-treatment with SA (10^4 μM) for different durations on survival of calli of ginger cv. Dadasiba exposed to CF of *F. oxysporum* f.sp. *zingiberi*[a]

SA treatment (h)	CF (ml)[b]							
	0		4		8		12	
	Per cent survival	Calli brown rating[c]	Per cent survival	Calli brown rating	Per cent survival	Calli brown rating	Per cent survival	Calli brown rating
0	100 (89)	1	57 (49)	2.9	3 (7)	4.0	0 (0.9)	4
12	100 (89)	1.2	97 (83)	1.5	80 (72)	2.3	80 (72)	2
24	100 (89)	1.2	87 (72)	1.8	87 (72)	2.2	83 (66)	2.2
48	90 (78)	2.1	83 (67)	2.5	83 (70)	2.2	67 (55)	2.7
72	100 (89)	1.8	80 (64)	2.9	73 (59)	2.5	63 (53)	2.3
LSD ($P \leq 0.05$)	Treatment duration	CF concentration						
Per cent survival	10.5	9.4						
Calli brown rating	0.3	0.3						

[a]Average of three replications with 10 calli per replication.
[b]CF = Culture filtrate added in 25 ml (v/v) callus culture medium.
[c]Calli brown rating; 1 = no browning, 2 = callus surface slightly brown, 3 = whole tissue brown, 4 = deep brown with restricted growth, 5 = deeply brown with no growth.
SA = Salicylic acid; values in parentheses are angular transformations; 0 = control.

of treatment of calli with SA. The rating of browning of calli increased with increase in SA treatment duration and was maximum in 48 and 72 h treatments on a medium without CF (Table 2). Insensitivity of the callus cultures to all the concentrations of CF increased significantly after prior treatment of calli with SA for 12 h (Table 2). Regardless of the CF dilutions, callus survival was maximum after 12 and 24 h treatment

with SA followed by 48 and 72 h durations of treatments. However, the mean rating for browning of calli were significantly lower in 12 and 24 h treatments (Table 2).

Enzyme activity in SA-treated calli

The effect of different concentrations of SA on peroxidase activity was studied in calli of ginger treated for 24 h. There was a sharp increase in peroxidase activity when concentration of SA increased from 10 to 100 μM. Maximum peroxidase activity was obtained in the calli treated with 10^4 μM concentration of SA (Figure 1). Further experiments were performed to determine the suitable duration of treatment of ginger calli with 10^4 μM SA for obtaining maximum peroxidase activity. Peroxidase activity increased in the calli treated with 10^4 μM SA for 8, 12 and 24 h over untreated calli (Table 3). However, there was no difference in peroxidase activity in calli treated for 4 h with SA compared to the control. The activity of β-1,3-glucanase was high in all SA treatments, being highest in calli treated for 8 h followed by 12 and 24 h treatments (Table 3). Though increased PAL activity was observed in calli treated with SA for 4 and 8 h, it was statistically similar to the control. The PAL activity dropped considerably after the 12 and 24 h treatments (Table 3).

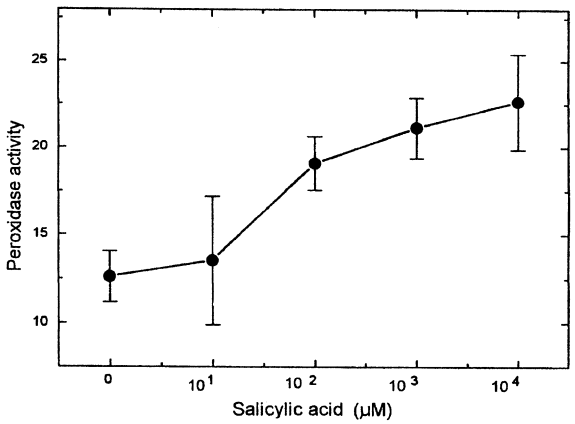

Figure 1. Peroxidase activity in calli of *Zingiber officinale* cv. Dadasiba treated with different concentrations of SA for 24 h (–•–) expressed as mean values of three replications. Bars represent standard error of mean. Peroxidase activity expressed as change in absorbance at 436 nm min^{-1} mg^{-1} protein was significantly affected by the concentration of SA ($P \leq 0.05$; LSD = 7.29).

Table 3. Effect of different durations of treatment with SA (10^4 μM) on enzyme activity in calli of ginger cv. Dadasiba[a]

Treatment duration (h)	Enzyme activity[b]		
	Peroxidase[c]	β-1,3-glucanase[d]	Phenylalanine ammonia lyase[e]
0	7.7	0.8	0.3
4	8.0	1.9	0.4
8	17.3	2.9	0.4
12	13.9	2.7	0
24	22	2.2	0
LSD ($P \leq 0.05$)	1.6	0.2	0.20

[a]Salicylic acid was added in callus culture medium.
[b]Values represent average of three replications.
[c]Peroxidase activity expressed as increase in absorbance at 436 nm min^{-1} mg^{-1} protein.
[d]β-1,3-glucanase activity expressed as mg glucose equivalents formed min^{-1} mg^{-1} protein.
[e]Phenylalanine ammonia lyase activity expressed as μM cinnamic acid formed min^{-1} mg^{-1} protein.

Induction of PR-proteins

The electrophoretic pattern of total soluble proteins extracted from calli treated with SA (10^4 μM) for different durations was performed on SDS-PAGE (Figure 2A). A protein band of approximately 97 kDa was observed in the extracts of callus cultures treated for 4, 8, 12 and 24 h (lanes 3–6) but not in the untreated calli (lane 2). SDS-PAGE also revealed another new protein band of 38 kDa in all the treatments which was absent in the untreated calli (lane 2). Higher intensity of a protein band of approximately 32 kDa was obtained in all the treatments (lanes 3–6) being very faint in the control (lane 2).

The total soluble proteins extracted from SA-treated (concentration of SA, 10^4 μM) calli were resolved by SDS-PAGE and transferred to nitrocellulose membrane for Western blotting. The membrane was incubated with McAb raised against tobacco PR-1 protein for immunodetection. The antiserum did not detect any band of 14 kDa protein (average size of PR-1 protein). However, the McAb cross-reacted with a 38 kDa protein band of protein extracts of calli treated for 4, 8 and 24 h with SA (lanes 2–4; Figure 2B).

In vitro *antifungal activity of PR-proteins*

The inhibitory effect of protein extracts obtained from calli treated with 10^4 μM SA for 0, 4, 8, 12 and 24 h was tested *in vitro*, by incubating the spore suspension

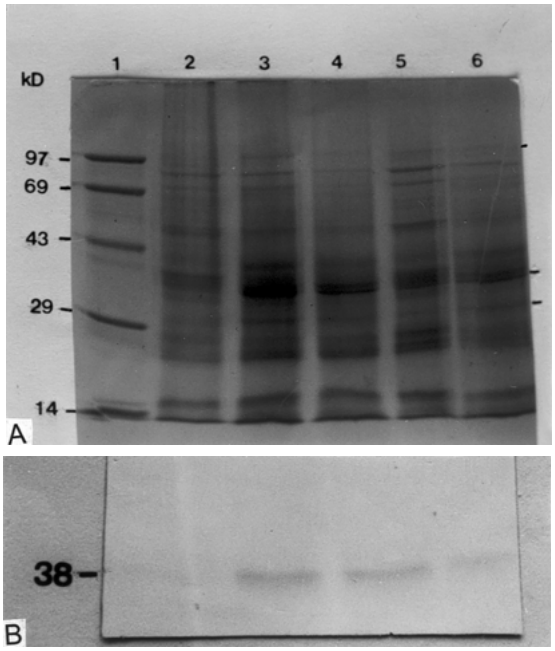

Figure 2. Electrophoretic analysis of PR-proteins in the calli of ginger, (A) SDS-PAGE analysis of PR-proteins obtained from calli treated with 10^4 μM SA for different durations. Lanes 2–6: protein profiles obtained after 0, 4, 8, 12 and 24 h of the treatment of callus cultures. 80 μg protein was added in each lane before electrophoresis. Gels were stained with coomassie blue G-250 (—) indicates the position of new protein band or bands with increased intensity. Lane 1: Molecular weight markers (Medium range, Bangalore Genei Pvt. Ltd. India), (B) Immunoblot of PR-proteins obtained from calli treated with 10^4 μM SA for different durations. 80 μg protein per lane was subjected to SDS-PAGE and immunoblotted with PR-1 monoclonal antibody. Lanes 1–4: proteins extracts obtained from calli treated for 0, 4, 8 and 24 h.

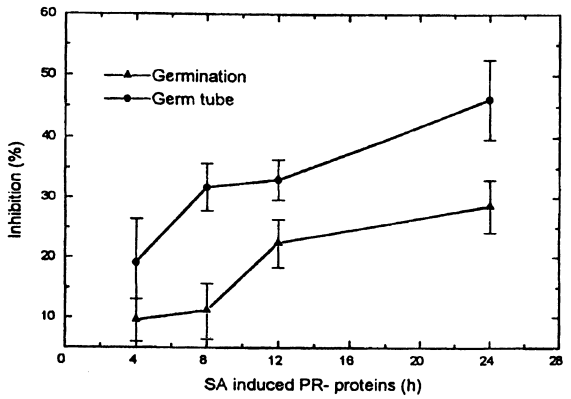

Figure 3. Effect of PR-proteins extracted from calli of ginger cv. Dadasiba treated with SA (10^4 μM) for different durations on inhibition of spore germination and germ tube length of *F. oxysporum* f.sp. *zingiberi* after 8 h of incubation. (–●–) values are mean of three replications with 100 spores/replication. Bars represent Standard error of mean. Per cent inhibition was significantly different for spore germination and germ tube length with LSD at ($P \leq 0.05$) of 8.98 and 10.10, respectively.

of *F. oxysporum* f.sp. *zingiberi* with protein extracts for 8 h. In general, with increase in duration of SA treatment of calli, there was a significant decrease in percentage spore germination and germ tube length (Figure 3). Similarly, a decrease in germ tube length of the spores was obtained in the protein extracts of all the treatments except from the 4 h treatment. Maximum inhibition in spore germination (28.7%) was obtained in protein extracts of calli treated for 24 h with SA. A similar trend was observed for inhibition in germ tube elongation (Figure 3).

Discussion

Several tolerance problems are associated with the application of SA on plants. Only a narrow safety margin separates the concentration at which this compound is effective in the induction of resistance and the rates at which it is strongly phytotoxic (Kessmann et al., 1994). Therefore, it was imperative to standardize a concentration of SA for the induction of insensitivity in the callus cultures of ginger without causing any phytotoxic effects. Experiments conducted with ginger calli indicated that treatment of calli with 10^4 μM SA for 12 and 24 h was most effective in the induction of insensitivity to CF of the pathogen. Treatment with SA for more than 24 h resulted in increased browning of calli, which may be due to its phytotoxic effect. Cai et al. (1996) also reported the use of a similar concentration of SA in rice seedlings for the induction of resistance to *Pyricularia grisea*.

SA-treated calli of ginger were also evaluated to see whether induction of insensitivity to CF was accompanied by an increase in the activity of peroxidase. Maximum increase in enzyme activity occurred in calli treated with 10^4 μM concentration of SA for 24 h. These calli were also insensitive to the CF of pathogen. The increase in activity are thus, directly correlated with the decreased sensitivity of SA-treated calli to the CF of *F. oxysporum* f.sp. *zingiberi*. Our results are in agreement with those of Anfoka and Buchenauer (1997), who reported increased peroxidase activity and induction of three new peroxidase isozymes in response to systemic acquired resistance (SAR) in tomato

against *Phytophthora infestans* by pre-inoculation with tobacco necrosis virus. SA-induced increase in peroxidase activity has also been reported in cucumber (Rasmussen et al., 1991) and barley (Tham and Huttova, 1996).

Peroxidases are a large family of enzymes with very diverse functions in plant systems. They often increase in response to stress. One of the principal roles of peroxidase enzyme appears to be cellular protection from reactive oxygen species generated by various stresses (Siegel, 1993). It was suggested that peroxidase may contribute to induced resistance to plant pathogens by generating H_2O_2 (Hammerschmidt, 1982) which has been reported to have an antifungal activity against various pathogens (Peng and Kuć, 1984). Increased peroxidase activity in resistance response has been related to lignification which has an important role in the reduction or blockage of nutrient diffusion from the neighboring host cells to the haustorium. Lignification also acts as a physical barrier to prevent the entry of fungal toxins and enzymes in the host systems (Kuć and Preisig, 1984; Conti et al., 1994). The results of the present study showed a direct correlation between increased peroxidase activity and its role in induction of insensitivity in callus cultures of ginger to CF of *F. oxysporum* f.sp. *zingiberi*. This may be due to the induced resistance responses like lignification that restrict the entry of fungal toxin in the cells of callus cultures.

In the present investigation, β-1,3-glucanase activity was increased after treatment of calli with SA. Renault et al. (1996) also demonstrated the induction of β-1,3-glucanase by SA treatment in grape leaves by immunoblotting. β-1,3-glucanase is a hydrolytic enzyme that is involved in the degradation of fungal cell walls (Boller, 1985).

Treatment of calli with SA did not show any significant increase in PAL activity. Rather a drop in the enzyme activity was found on prolonged incubation of calli on MS medium containing SA (10^4 μM). In rice – *Xanthomonas campestris* pv. *oryzae* and kiwi – *Sclerotinia sclerotiorum* systems, SA-induced SAR has been reported to be due to enhanced PAL activity (Xiao et al., 1996; Reglinski et al., 1997). However, a major function of PAL is to produce precursors for the synthesis of SA in plant defense mechanisms (Silverman et al., 1995; Mauch-Mani and Slusarenko, 1996).

In our study, electrophoretic analysis of total soluble proteins extracted from calli treated with SA (10^4 μM) for different durations resulted in the induction of new protein bands of ∼97 and 38 kDa. Presence of a new protein band upon treatment with SA has also been obtained in tomato – *Phytophthora infestans* system (Christ and Mosinger, 1989). An increased intensity of a protein band of ∼32 was also obtained in ginger calli treated with SA for various durations. However, these differences were only quantitative between treated and untreated calli. These proteins appear to be constitutively present in the calli. PR-proteins have also been identified and classified in *Rhizoctonia*-infected rice leaf sheaths (Bera and Purkayastha, 1997), where out of the 16 proteins identified, six were constitutively present and six were formed *de novo*. In the present study, *de novo* synthesis of proteins could not be detected.

Western blotting and immunodetection of PR-proteins with McAb raised against tobacco PR-1 proteins detected a 38 kDa band in SA-treated calli. These results suggest that antibody for tobacco PR-1 shared a common antigenic site with the 38 kDa ginger PR-protein, although these displayed a marked difference in their molecular weights. However, it may not be PR-1, since, all PR-1 proteins identified till date are about 14 kDa (Malamy and Klessig, 1992). Various PR-proteins accumulate in response to SA-induced SAR response in a broad range of monocotyledonous and dicotyledonous plants. However, all plant-pathogen systems do not respond to SA treatment (Malamy and Klessig, 1992). It is clear from the present study that exogenous application of SA in the callus cultures of ginger induces PR-proteins which can be separated by electrophoresis and identified by immunodetection by use of monoclonal antibodies.

Total proteins extract of SA-treated calli was tested for their *in vitro* antifungal activity against *F. oxysporum* f.sp. *zingiberi*. Inhibitory effects of PR-proteins on spore germination and germ tube elongation were obtained.

The findings of this study reveal another example (ginger – *F. oxysporum* f.sp. *zingiberi*) of SA-induced resistance which is correlated with biochemical analysis of the insensitivity response. Induction of PR-proteins, their immunodetection and antifungal activity of PR-proteins obtained during this investigation is a maiden attempt in ginger – *F. oxysporum* f.sp. *zingiberi* system. Whether this SA-induced insensitivity of callus cultures of ginger to *F. oxysporum* f.sp. *zingiberi* is akin to induced SAR in ginger plants towards *F. oxysporum* f.sp. *zingiberi* is being examined.

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