



Induced resistance in bhendi against powdery mildew by foliar application of salicylic acid

R.Vimala and M.Suriachandraselvan

ABSTRACT

The effect of salicylic acid in inducing systemic resistance in bhendi against powdery mildew caused by *Erysiphe cichoracearum* DC was investigated by the application of salicylic acid (1mM) to induce production of defense related enzymes and chemicals in plants when it was applied as pre-inoculation, post-inoculation, salicylic acid alone, pathogen alone and water control. The results revealed that earlier and increased activities of phenyl alanine ammonia lyase, was observed in salicylic acid pretreated bhendi plants challenge inoculated with *E.cichoracearum*. Higher accumulation of phenolics was also noticed in plants pretreated with salicylic acid and able to enhance the resistance against invasion of *E. cichoracearum* in bhendi.

Key words: Salicylic acid, induced systemic resistance, bhendi powdery mildew

INTRODUCTION

Resistance to pathogens is associated with the accumulation of enzymes, antibiotics and inhibitors. It can be induced in the host through the application of salicylic acid, which is the most important signal molecule. Salicylic acid is a natural phenolic compound present in many plants and is an important component in the signal transduction pathway and is involved in local and systemic resistance to pathogens (Delaney *et al.*, 1995 and Maleck *et al.*, 2000). With increasing awareness of possible deleterious effects of fungicides on the ecosystem and growing interest in pesticide free agricultural products, biological control now appears to be a promising strategy for managing diseases in a range of crops (David, 2008). The application of exogenous salicylic acid was shown to mimic certain aspects of a pathogen infection resulting in induction of resistance (Vernooij *et al.*, 1995; Glazebrook, 2001). Several attempts have been made to induce resistance in some plants by exogenous application of salicylic acid (Padmaja *et al.*, 2004). In the present study the effect of salicylic acid in inducing systemic resistance in bhendi against powdery mildew caused by *Erysiphe cichoracearum* was investigated.

MATERIALS AND METHODS

Method of induction

To study the effect of salicylic acid on changes in phenolics and phenylalanine ammonia-lyase in bhendi leaves, bhendi plants (Arka Anamica variety) of thirty days

old were sprayed with salicylic acid (1mM) as pre-pathogen inoculation and post-pathogen inoculation. Water suspensions of powdery mildew conidia were prepared by scrapping the conidia from the infected bhendi leaves and were sprayed on healthy leaves. Pathogen alone inoculated plants served as control. Inducer alone treated and water sprayed plants were maintained for comparisons. At various times (0 to 7 days) after treatments leaf samples were collected and various analyses were made.

Estimation of phenolic content

Total phenol content of the bhendi leaf was estimated by Folin Ciocalteu method (Bray and Thorpe, 1954). One gram of leaf sample was homogenized in 10 ml of 80 per cent ethanol and agitated for 15 minutes at 70°C; filtered through muslin cloth and again through Whatman No.1 filter paper and the volume of the filtrate was adjusted to 5 ml with 80 per cent ethanol. In a test tube, one ml of ethanol extract, one ml of Folin Ciocalteu reagent and 2 ml of 20% sodium carbonate solution were added and the mixture was heated in a boiling water bath for a minute. Then the tube was cooled under running tap water. After cooling, the volume was made up to 25 ml with distilled water. A reagent blank was maintained with one ml of distilled water instead of leaf extract. The intensity of colour was read at 650 nm in a calorimeter. The amount of total phenols present in the sample was calculated from a standard curve prepared by using different concentrations of catechol.

Estimation of phenylalanine ammonia lyase (PAL) activity

One gram of the leaf sample was homogenized in three ml of ice-cold 0.1M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1g of insoluble polyvinylpyrrolidone. The extract was filtered through cheese cloth and the filtrate was centrifuged at 15000g for 15 minutes. The supernatant was used as enzyme source. Phenylalanine ammonia lyase activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm (Dickerson *et al.*, 1984). Sample containing 0.4 ml of enzyme extract was incubated with 0.5ml of 0.1M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 minutes at 30°C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 M⁻¹ cm⁻¹. Enzyme activity was expressed as synthesis of trans-cinnamic acid (in nmol quantities) min⁻¹ g⁻¹ fresh weight.

Statistical analysis

The data were subjected to statistical scrutiny following the method of Panse and Sukhatme (1989) and Gomex and Gomex (1984) and the means were separated by Duncan's Multiple Range Test (DMRT).

RESULTS**Phenolic content of bhendi leaves**

All the treatments except control increased the total phenol content in bhendi leaves (Table 1). Pre-inoculation spray of salicylic acid showed the maximum phenolic content followed by post-inoculation spray. Pathogen alone or salicylic acid alone also increased the phenolic content but the level was lesser when compared to pre and post-inoculation spray of salicylic acid. The phenolic content increased up to four to five days after treatment and thereafter a decreasing trend was observed. But even on seventh day after treatment, the total phenol content

was more in all the treatments than the initial level i.e., the control on zero day.

Phenylalanine ammonia lyase activity of bhendi leaves

The results on changes in Phenylalanine ammonia lyase (PAL) activity due to foliar application of salicylic acid are presented in table 2. Phenylalanine ammonia lyase activity ranged from 57.72 to 140.60. The results revealed that there was an increase in PAL activity due to treatments. The increase was maximum in pre-inoculation spray followed by post-inoculation spray, whereas in pathogen alone and in salicylic acid alone treated plants, the activity was comparatively lesser i.e. 97.64 and 96.91 respectively. Phenylalanine ammonia lyase activity reached the maximum on fourth day after treatment and showed a declining trend thereafter. But the activity was higher than the initial level throughout the experimental period.

DISCUSSION**Phenol**

The studied abiotic inducer namely salicylic acid brought significant increase in phenol content of pre-inoculation spray of the salicylic acid resulted in higher increase in the phenol level than in post-inoculation spray. Phenol content increased steadily from the day of treatment and the increase continued up to fourth day. Similar observations on other host-pathogen combinations as influenced by abiotic elicitor treatments have been reported earlier. For instance, Meena *et al.* (2001) found that salicylic acid applied as pre-inoculation spray in groundnut plants challenge inoculated with *Cercosporidium personatum* resulted in three fold increase in phenol content on fourth day. Accumulation of phenolics at the site of infection is a general response of plants in many host-pathogen interactions (Farkas and Kiraly, 1962) and this accumulation is fostered by biotic

Table 1. Effect of foliar application of salicylic acid on phenolic content (mg g⁻¹) in bhendi leaves

Treatments	Days after treatment								Mean
	0	1	2	3	4	5	6	7	
Pre-inoculation spray	181.52 ^a	300.92 ^a	316.80 ^a	393.33 ^a	421.63 ^a	410.60 ^a	360.90 ^a	326.54 ^a	339.03 ^a
Post- inoculation spray	180.26 ^{ab}	296.33 ^b	306.90 ^b	375.62 ^b	410.80 ^b	389.33 ^b	327.52 ^b	305.22 ^b	323.99 ^b
Salicylic acid alone	180.00 ^b	290.52 ^d	297.54 ^d	355.33 ^d	365.30 ^d	383.22 ^d	297.80 ^c	280.55 ^d	306.28 ^d
Pathogen alone	177.84 ^c	292.43 ^c	298.92 ^c	360.90 ^c	368.63 ^c	385.06 ^c	295.44 ^d	284.64 ^c	307.98 ^c
Distilled water	175.16 ^d	177.33 ^e	179.66 ^e	177.86 ^e	178.52 ^e	181.36 ^e	179.36 ^e	175.90 ^e	178.14 ^e
Mean	178.96 ^A	271.51 ^B	279.96 ^D	322.61 ^F	341.98 ^G	349.91 ^H	292.20 ^E	274.57 ^C	

In the column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 2. Effect of foliar application of salicylic acid on PAL activity (h_{mol} trans-cinnamic acid min⁻¹g⁻¹) in bhendi leaves

Treatments	Days after treatment								Mean
	0	1	2	3	4	5	6	7	
Pre-inoculation spray	61.33 ^a	105.23 ^a	122.40 ^a	130.30 ^b	140.60 ^a	122.86 ^b	90.80 ^a	87.20 ^b	107.59 ^a
Post-inoculation spray	60.93 ^a	101.83 ^b	115.16 ^b	121.20 ^b	135.66 ^b	121.93 ^c	89.13 ^b	86.06 ^a	103.99 ^b
Salicylic acid alone	59.00 ^b	92.52 ^d	104.60 ^d	113.32 ^c	120.90 ^d	130.70 ^a	78.82 ^d	75.40 ^b	96.91 ^d
Pathogen alone	58.06 ^c	97.16 ^c	108.20 ^c	114.20 ^c	132.20 ^c	114.63 ^d	80.40 ^c	76.26 ^b	97.64 ^c
Distilled water	57.72 ^c	58.24 ^c	57.50 ^e	59.80 ^d	58.70 ^e	57.32 ^e	58.54 ^e	56.80 ^e	58.08 ^e
Mean	59.41 ^H	91.00 ^E	101.57 ^D	107.76 ^C	117.61 ^A	109.49 ^B	79.54 ^F	76.34 ^G	

In the column, means followed by a common letter are not significantly different at 5% level by DMRT

and abiotic elicitors. Retig and Chet (1974) also found a marked accumulation of total phenols when tomato plants were fed with catechol and this resulted in suppression of symptom expression due to infection by *Fusarium oxysporum* f.sp. *lycopersici*. Matern and Kneusal (1988) expressed the view that the first stage of defense in plants is the accumulation of phenols at the infection site which restricts the growth of the pathogen. Systemic acquired resistance (SAR) is characterized in plants such as Arabidopsis by the marked reduction in susceptibility to disease resulting from prior infection with an avirulent, necrotizing pathogen. Induction of SAR is dependent largely on the hormone salicylic acid (SA), which is essential for the activation of SAR genes and gene-for-gene resistance, and for limiting disease caused by virulent pathogens (Mauch-Mani and Metraux, 1998).

Phenylalanine ammonia lyase (PAL)

PAL is the key enzyme of phenylpropanoid metabolism in higher plants which catalyzes the conversion of phenylalanine to trans-cinnamic acid which supplies the precursors for flavanoids, lignins and phytoalexins (Hahlbrock and Scheel, 1989). Several studies indicated that the activation of PAL and subsequent increase in phenolic content in plants is a general response associated with disease resistance (Velazhahan and Vidhyasekaran, 1994). In the present study, pre-inoculation spray of salicylic acid showed increase in PAL activity from the time of treatment. Thulke and Conrath (1998) stated that pre-treatment with salicylic acid or functionally related inducers such as 2,6 dichloroisonicotinic acid or benzothiadiazole induced hydrogen peroxide generation or expression of defense related genes such as PAL or 4 coumarate CO A ligase. Phosphate treatment of first leaf of barley led to significant increase in activity of PAL in second leaf (Mitchell and Walters, 2004). Systemic acquired resistance is

accompanied by expression of a diverse array of defence genes (many encoding pathogenesis-related proteins) and enhanced broad-spectrum disease resistance throughout the plant (Maleck *et al.*, 2000; Glazebrook, 2001). Previously it was reported that certain biochemicals such as sugars and potassium are closely associated with the resistance to powdery mildew in mungbean (Gawande and Patil, 2004). Our results showed that salicylic acid also induced this kind of activity. However it was recommended of water extracts produced from rice straw (RST) and empty fruit bunch of oil palm (EFB) composts fortified with *Trichoderma harzianum* for the control of Choanephora wet rot of okra caused by *Choanephora cucurbitarum* was studied under field conditions (Siddiqui *et al.*, 2008). Our results mainly concern with the inducement of resistance. Combination of these methods can be utilized for the protection of okra.

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R.Vimala* and M.Suriachandraselvan

Cotton Research Station, Tamilnadu Agricultural University, Srivilliputtur - 626 125, Tamil Nadu, India,
 *Communication author E-mail: ramesh_vimala@yahoo.co.in