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Host sensitization by phytoalexin inducers : I. Induction of resistance in barley seedling against *Helminthosporium sativum* by seed treatment with reducing agents

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Out of five reducing agents tested at 10^{-3} M to 10^{-5} M for seed treatment, chemicals at their effective concentration induced appreciable resistance in barley seedling to *Helminthosporium sativum* and caused 30 to 70 per cent reduction in disease incidence when inoculated at the age of 3 weeks. Stronger effects were recorded with thioglycollic acid, cysteine and cysteine-HCL at 10^{-3}M concentration. All the chemicals at their effective concentration reduced the number of lesions and some of them inhibited lesion expansion also. The effect of treatment also persisted at a decline mode when the seedlings were inoculated at the age of 38 days. *In vitro* toxicity of the chemicals did not seem to be related with the induction of resistance.

Key words : Relative effects, reducing agents, barley seedling, induction of resistance

INTRODUCTION

Role of phytoalexin in disease resistance of different plants is being increasingly realized. Different groups of chemicals have the ability to induce phytoalexin production in different plant species (Perrin and Cruickshank, 1965; Bell, 1967; Schwochau and Hadwiger, 1968; Cheema and Hard, 1978). The possibility of such chemicals in the induction of resistance has been successfully explored in rice and wheat (Sinha and Giri, 1979; Sinha and hait, 1982; Giri and Sinha, 1983a and 1983b; Hait and Sinha, 1986; Gangulee and Sinha, 2003). Good correlation also exists between the induction of resistance with the induced production of post-infectionally produced phytoalexin-like substance (Sinha and Hait, 1982; Hait and Sinha, 1986). Later, similar effect has been also observed in tomato and soybean (Mandal and Sinha, 1992 : Mitra and Choudhury, 2001). The presence of phytoalexin-like antifungal substance in barley has been reported earlier (Scott *et al.*, 1957; Oku *et al.* 1975 a ; b). Further studies has been undertaken to explore the possibility of phytoalexin inducing chemicals to induce resistance in barley against infection by *Helminthosporium sativum* Pammel, King and Bakke.

MATERIALS AND METHODS

A highly susceptible cultivar of barley P-486 was the experimental material used. Five reducing agents known to induce phytoalexin in plants (Perrin and Cruickshank, 1965; Schwochau and Hadwiger, 1968; Sinha and Hait, 1982; Hait and Sinha, 1986) were used as seed treatment. All the chemicals were used at 10^{-3}M to 10^{-5}M concentration. The effect of chemicals on spore germination was done following Giri and Sinha (1983a). Seeds were soaked in different concentrations or in distilled water as control before sowing in soil supplemented with dried powder of well rotten farmyard manure in 14 cm earthen pots. The pots were kept in sunlight and watered daily. The plant population was reduced by thinning before inoculation so as to keep about thirty seedlings per pot. Seedlings raised from treated and untreated seeds were inoculated at the age of 3 and 5 weeks by spraying on leaves with the conidial suspension of virulent isolate of *Helminthosporium sativum* at 5×10^5/ml concentration, grown on PDA medium in 8 cm diameter Petridishes at 28°C for 6 days. The treated and untreated plants in separate pots were sprayed with water as uninoculated control. Plants after inoculation were kept in a humid chamber for 12 hrs to fa-

cilitate infection and then kept in sunlight again. Disease assessment was done based on symptoms developed on leaves 4 days after inoculation (Sinha and Das, 1972) taking into consideration both in size and number of lesions. Disease index was recorded as the mean of forty five plants, fifteen from each pot selected randomly. To obtain diffusates, leaves were collected from plants of different treatments in inoculated and uninoculated condition after 72 hrs of inoculation. Leaves were collected from differeent sets and cut into small pieces (about 2.5 cm long), washed in sterile distilled water and blotted dry with blotting paper. Leaves were then placed in sterile tube (8×2 cm diameter) with 5 ml of sterile distilled water and incubated for 24 hrs at 27°C. After collection, the diffusates were passed through sintered glass filter to make cell free and then assayed for their effect on germination of conidia of *H. sativum* on groove slide following Sinha and Trivedi, (1978). The direct effect of the chemicals on conidial germination was also similarly assayed.

RESULTS AND DISCUSSION

The chemicals were tested for its toxic effect, if any, on spore germination and germ tube growth of the pathogen. The five reducing agents, sodium selenite, sodium sulphite, thioglycollic acid, cysteine and cysteine-HCL were tested at 10^{-3}, 10^{-4} and 10^{-5} Molar (Table 1). Thioglycollic acid and sodium selenite at 10^{-3} M showed much toxicity both on germination and germ tube growth, but the other sodium sulphite, cysteine and cysteine-HCL were less toxic. The direct toxicity of the chemicals gradually declined in all the cases both on spore germination and germ tube growth. Thioglycollic acid at 10^{-5} M and cysteine at 10^{-4} M and 10^{-5} M though had some toxic effect on spore germination but it enhanced tube length much more than the control. Other three chemicals showed little to moderate effect on germ tube length.

Initially five chemicals were tested at three concentrations each for their possible effect of seed treatment, if any, in inducing resistance in barley seedlings to *Helminthosporium* infection from which they suffered at times with considerable damage. Seedlings developed from treated seeds at different concentrations did not show any phytotoxic effect (Table 2). With the exception of sodium sulphite, all other four chemicals showed 19-70% reduction of disease at their 10^{-3}M, but the effects reduced with dilution. Sodium sulphite (10^{-4}M) showed 42% reduction of disease and its effect become reduced at both in higher and lower concentrations. Reduction of lesion numbers by 6-67% was recorded at the effective concentration of different chemicals. Reduction of disease mostly by reducing lesion numbers was recorded in cysteine and cysteine-HCL and by reducing lesion size by thioglycollic acid and sodium sulphite at their effective concentration. Sodium selenite at 10^{-3}M caused reduction of disese both by reducing number and size of lesions.

Table 1 : Effect of five reducing agents at different concentrations on spore germination and germ tube growth on *Helminthosporium sativum.***

Chemicals	Conc. (M)	Germinating percentage.*	Mean germ tube length#
Water (Control)		100.00	100.00
Sodium selenite	10^{-3}	15.8	5.5
	10^{-4}	49.8	12.1
	10^{-5}	73.9	68.7
Sodium sulphite	10^{-3}	40.5	36.1
	10^{-4}	47.8	59.2
	10^{-5}	70.8	90.5
Thioglycollic acid	10^{-3}	7.5	52.7
	10^{-4}	41.7	82.2
	10^{-5}	55.1	104.6
Cysteine	10^{-3}	36.2	26.7
	10^{-4}	34.2	108.6
	10^{-5}	76.5	126.8
Cysteine-HCL	10^{-3}	39.9	30.4
	10^{-4}	47.3	50.3
	10^{-5}	69.5	85.4

** Results expressed as percentage in terms of control.
* Average of 300 spores ; # Average of 60 germlings.

Table 2 : Effect of wet seed treatment with five reducing agents, used at three concentrations, on symptom expression in pot-grown barley seedling inoculated with *Helminthosporium sativum* after 3 weeks

Chemicals	Conc. (M)	Mean no. of lesion/plant.*	Mean disease index/plant.*
Water (Control)		27.8	9.5
Sodium selenite	10^{-3}	20.6(-25.9)	4.6(-51.6)
	10^{-4}	30.8(+10.8)	6.6(-30.5)
	10^{-5}	38.2(+37.4)	10.5(+10.5)
Sodium sulphite	10^{-3}	34.1(+22.7)	7.7(-18.9)
	10^{-4}	22.8 (-18.0)	5.5(-42.1)
	10^{-5}	26.0(-6.0)	5.7(-40.0)
Thioglycollic acid	10^{-3}	21.1(-24.1)	3.2(-66.3)
	10^{-4}	33.4(+20.1)	6.3(-33.7)
	10^{-5}	29.4(+5.8)	6.7(-29.5)
Cysteine	10^{-3}	11.1(-60.1)	3.1(-67.3)
	10^{-4}	25.4(-8.6)	6.4(-32.6)
	10^{-5}	23.6(-15.1)	6.7(-29.5)
Cysteine-HCL	10^{-3}	9.1(-67.2)	2.8(-70.5)
	10^{-4}	28.2(+01.4)	6.6(-30.5)
	10^{-5}	28.0(+0.7)	8.7(-8.4)
C.D(P=0.05)			3.39
C.D(P = 0.01)			4.90

* Values in parenthesis indicate percentage reduction/increase in

Table 3 : Effect of seed soaking with four selected chemicals on *Helminthosporium sativum* infection in pot grown barley plants (cv. P-486) after 3 and 5 weeks and spore germination in leaf diffusates from uninoculated and inoculated plants.

Chemicals	Conc.	Mean germ tube growth (μ) in leaf diffusate †			Mean no. of spots/plant*	Mean disease index/plant*	Mean germ tube growth (μ) in leaf diffusate †		Mean no. of spots/plant*	Mean disease index/plant*
		Uninoculated		Inoculated			Uninoculated	Inoculated		
		14 days	24 days	24 days			38 days	38 days		
Water (Control)		28.3	66.0	58.7 (–11.1)	32.9	8.6	61.5	54.2 (–11.8)	39.8	9.4
Thioglycollic acid	10^{-3}	7.9 (–71.4)	41.5 (–37.1)	31.6 (–52.1)	24.6 (–25.2)	2.8 (–67.4)	47.4 (–22.9)	41.1 (–33.2)	35.0 (–12.1)	6.1 (–37.8)
Sodium selenite	10^{-3}	10.0 (–64.5)	51.2 (–22.4)	35.5 (–46.2)	24.0 (–27.1)	4.1 (–52.3)	55.0 (–10.6)	45.6 (–25.9)	30.0 (–24.6)	7.3 (–25.5)
Cysteine	10^{-3}	12.1 (–57.2)	53.2 (–19.4)	32.3 (–51.0)	13.1 (–60.2)	2.7 (–68.6)	56.1 (–8.7)	41.2 (–33.0)	31.0 (–22.1)	6.2 (–36.7)
Cysteine-HCl	10^{-3}	12.6 (–55.5)	52.5 (–20.5)	32.0 (–51.5)	10.5 (–68.1)	2.6 (–69.7)	55.5 (–9.8)	42.9 (–30.2)	29.8 (–25.1)	6.6 (–32.6)
C.D (P=0.05)		1.50				2.41				1.80
C.D (P-0.01)		2.07				3.52				2.48

C.D to compare between any two means of germ tube growth (P=0.05) = 6.39 (24 days) 5.54 (38 days).
C.D to compare between any two means of germ tube growth (P=0.05) = 10.63 (24 days) 9.20 (38 days).
* Values in parenthesis indicate percentage reduction in terms of control in case of disease index and in terms of uninoculated control in case of germ tube growth.
† Results represented in terms of sixty observations.

terms of control.

Table 4 : Effect of seed soaking with four chemicals on *Helminthosporium* infection by natural inoculum in field grown barley plants after 3 weeks

Chemicals	Conc. (M)	Mean no. of spots/plant.*	Mean disease index/plant.*
Water (Contro		14.4	5.2
Thioglycollic acid	10^{-3}	9.1(-36.8)	2.2(-55.2)
Sodium selenite	10^{-3}	11.0(-23.6)	2.7(-48.1)
Cysteine	10^{-3}	8.5(-40.9)	2.5(-51.9)
Cysteine-HCL	10^{-3}	8.2(-43.1)	2.5(-51.9)
C.D(P=0.05)			1.12
C.D(P=0.01)			1.56

* Values in parenthesis indicate percentage reduction in terms of control.

In the next experiment four effective chemicals with their effective concentration were selected and further tested (Table 3). Plants grown by different treatments were inoculated at 3 and 5 weeks. Leaf diffusates were collected initially from 14 days old uninoculated plants and after 24 and 38 days in different treatments, from both inoculated and uninoculated control i.e. 3 days after inoculation and bioassayed for fungitoxicity. All the chemicals, used for seed treatment effectively protected the seedling at 3 week stage against *H. sativum* infection. The chemicals showed appreciable control of disease ranged between 52-69% and reduction of lesion numbers by 25-68%. Reduction of disease mostly by reducing lesion numbers was recorded in cysteine and cysteine-HCL and reducing number and size of lesions by thioglycollic acid and sodium selenite. The effect of the chemicals was reduced in all the 4 treatments showing 25-37% reduction of disease. Reduction of disease in all the 4 treatments took place due to reduction of both size and number of lesions. The effect of leaf diffusates of 14 days old plant in different treatments showed 55-71% reduction on germ tube growth gradually reduced with age of the plant. On inoculation the treated plant showed production of fungitoxic substance at a very significant level.

Field trial with effective chemicals (Table 4) at their effective concentration was tested against natural inoculum of *H. sativum*. All the chemicals showed appreciable control of disease ranged between 48-55% and reduction of lesion numbers by 24-43%. The reduction of disease caused by reduction of lesion size as well as lesion numbers.

All the five reducing agents used for wet seed treatment provided effective protection to 3 week old barley seedlings against inoculation with *H. sativum*. Less, but still considerable, effects were observed in different treatments involving the more effective compounds when plants were inoculated 2 weeks later. Seed treatments interfered with the infection process itself, since the effective chemicals caused significant reductions in the number of lesions, particularly at 3 weeks old plants. But in some of the treatments at their effective concentration, there were evidence of reduction of mean le-

sion size implying appreciable inhibition of lesion enlargement at this stage. Similar effects were also pronounced in older plants i.e. at 5 weeks.

Moderate to high levels of fungitoxicity observed in the diffusates from 2 week old barley seedlings in different treatments rapidly declined with age. While untreated plants developed considerable fungitoxicity when inoculated after 3 or 5 weeks, those in most treatments developed more toxicity under similar conditions. The greater amount of post-infectionally developed fungitoxicity in the treated plants, even 5 weeks after seed treatment, appeared to be more significant than the initial development of toxicity in them. The above result might have caused from the interaction between seed treatment and infection, mediated possibly through some alteration in host metabolism. Some of the chemicals were also effective to induce resistance at significant level against natural infection in field condition.

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