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Host sensitization by phytoalexin inducers : 2. Induction of resistance in barley seedling against *Helminthosporium sativum* by seed treatment with metabolic inhibitors

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Out of five metabolic inhibitors, sodium malonate, sodium fluoride, sodium azide and sodium iodoacetate were tested at 10^{-3}M to 10^{-5}M and cycloheximide tested at 10^{-4}M to 10^{-6}M for seed treatment. Chemicals at their effective concentration induced appreciable resistance in barley seedling to *Helminthosporium sativum* and caused 33 to 69 per cent reduction in disease incidence when inoculated at the age of 3 weeks. Stronger effects were recorded with cycloheximide and sodium azide at 10^{-4}M and both sodium malonate and sodium iodoacetate 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 35 days. *In-vitro* toxicity of the chemicals does not seem to be related with the induction of resistance.

Key words: Barley seedling, metabolic inhibitors, relative effects, induction of resistance.

INTRODUCTION

The active role of phytoalexins in disease resistance of many plants is being increasingly realized. Chemicals of different groups have the ability in inducing phytoalexin production in different plant species (Perrin and Cruickshank, 1965; Bell, 1967; Schwochua and Hadwiger, 1968; Cheema and Hard, 1978). The possibility of such group of 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, 1983 a and b; Hait and Sinha, 1986; Gangulee and Sinha, 2003). Significant correlation also exists between the induction of resistance and the induced production of post-infectionally produced phytoalexin- like substance (Sinha and Hait,1982; Hait and Sinha, 1986). Similar effect is also observed in tomato, soybean and barley (Mandal and Sinha, 1992; Mitra and Choudhury, 2001; Hait, 2008). The production of phytoalexin-like antifungal substance in barley has been reported earlier (Scott *et al.,* 1957; and Oku *et al.* 1975a and b). Further studies has been undertaken to explore the possibility of other phytoalexin inducing chemicals to induce resistance in barley against *Helminthosporium sativum* Pammel, King and Bakke infection.

MATERIALS AND METHODS

Highly susceptible cultivar of barley, P-486 was the experimental material used. Five metabolic inhibitors, sodium malonate, sodium fluoride, sodium azide, sodium iodoacetate, cycloheximide were known to induce phytoalexin in plants and were used as seed treatment. Out of five metabolic inhibitors, four were tested at 10^{-3}M to 10^{-5}M and another one i.e. cycloheximide tested at 10^{-4}M to 10^{-6}M for seed treatment. The effect of chemicals on germination of spore was done following Giri and Sinha, (1983a). Seeds were soaked in different concentrations of chemicals or in distilled water as control before sowing in soil with the addition of 100 g of dried powder of well rotten farmyard manure in 12 cm earthen pots. The pots were kept in sunlight and watered daily as per requirement. The plant population was reduced by thinning before inoculation so as to keep about twenty five to thirty seedlings per pot. Seedlings raised jfrom 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 $x10^5$ conidia/ml concentration. For the production of conidia, the pathogen was grown on PDA medium in 8 cm diameter Petridishes at 28^0C for 6 days. As

uninoculated control, the treated and untreated plants in separate pots were sprayed with water. Plants after inoculation were kept in a humid chamber for 6 hrs to facilitate 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 number and size of lesions. Disease index was done as the mean of forty five plants, fifteen from each pot selected randomly. To obtain diffusates, both inoculated and uninoculated leaves were collected from plants of different tretments after 72 hrs of inoculation. Two gram leaves were collected from different sets and cut into small pieces, each about 2.5 cm long. Leaves were washed in sterile distilled water and blotted dry with sterile blotting paper. Leaves were then placed in sterile tube (8 x 2 cm diameter) with 5 ml of sterile distilled water, covered with cap and then 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

The chemicals were tested initially for its toxic effect, if any, on spore germination and germ tube growth of the pathogen, *H. sativum*. Out of five metabolic inhibitors, four i.e sodium malonate, sodium fluoride, sodium azide and sodium iodoacetate were tested at 10^{-3}M to 10^{-5}M and cycloheximide was tested at 10^{-4}M to 10^{-6}M (Table 1). Cycloheximide at 10^{-4}M and sodium azide at 10^{-3}M conc. showed much toxicity both on germination and germ tube growth, but the other three sodium malonate, sodium fluoride and sodium iodoacetate were less toxic. The direct toxicity of the metabolic inhibitors gradually declines in alll the cases both on spore germination and germ tube growth but the effect of both cycloheximide at 10^{-6}M and sodium azide at 10^{-5}M still persisted. 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). All the chemicals showed 33-54% reduction of disease at their 10^{-3}M concentration and cycloheximide caused 69% reduction at 10^{-4}M, but the effects became reduced with dilution. Sodium azide (10^{-4}M) showed 53% reduction of disease and its effects became reduced at both in higher and lower concentrations. Reduction of lesion numbers by 22-67% was recorded at the concentrations used in different chemicals but it was insignificant in sodium iodoacetate at 10^{-5}M concentration. Reduction of disease mostly by reducing lesion numbers was recorded in sodium malonate and sodium fluoride and by reducing lesion expansion by cycloheximide, sodium azide and sodium iodoacetate at their effective concentration. Sodium iodoacetate at 10^{-4}M caused reduction of disease both by reducing number and size of lesions.

In the next experiment of the series, 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

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

Chemicals	Conc. (M)	Germinating percentage.*	Mean germ tube length (μm)
Water (Control)		100.00	100.00
Cycloheximide	10^{-4}	16.9	20.9
	10^{-5}	30.1	38.1
	10^{-6}	39.3	45.1
Sodium malonate	10^{-3}	64.1	42.1
	10^{-4}	73.6	60.3
	10^{-5}	78.0	62.3
Sodium fluoride	10^{-3}	66.3	21.1
	10^{-4}	74.7	39.8
	10^{-5}	81.5	47.6
Sodium azide	10^{-3}	37.0	38.8
	10^{-4}	39.2	43.8
	10^{-5}	41.4	47.4
Sodium iodoacetate	10^{-3}	44.8	52.0
	10^{-4}	54.4	66.8
	10^{-5}	61.6	78.4

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

weeks. Leaf diffusates were collected initially from 14 days old uninoculated plants and after both 24 and 38 days in different treatments, from both inoculated and unionoculated control i.e. 72 hrs after inoculation and these were bioassayed for fungitoxicity. All the chemicals, used for seed treatment effectively protected the seedling at 3-week stage against *Helminthosporium sativum* infection. The chemicals showed appreciable

Table 2 : Effect of wet seed treatment with five metabolic inhibitors, 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)		24.5	7.1
Cycloheximide	10-4	10.7 (-56.3)	2.2 (-69.1)
	10-5	8.1 (-66.9)	3.9 (-45.1)
	10-6	14.8 (-39.6)	4.1 (-42.3)
Sodium malonate	10-3	10.5 (-57.1)	3.3 (-53.5)
	10-4	16.1 (-34.3)	3.4 (-52.1)
	10-5	15.2 (-37.9)	4.3 (-39.4)
Sodium fluoride	10-3	15.8 (-35.5)	4.7 (-33.8)
	10-4	19.0 (-22.4)	5.4 (-23.9)
	10-5	15.8 (-35.5)	4.8 (-32.4)
Sodium azide	10-3	13.3 (-45.7)	3.7 (-47.9)
	10-4	16.6 (-32.2)	3.3 (-53.5)
	10-5	18.1 (-35.5)	4.0 (-43.7)
Sodium iodoacetate	10-3	15.9 (-35.1)	3.4 (-52.1)
	10-4	19.1 (-22.1)	4.7 (-33.8)
	10-5	24.3 (-0.8)	6.3 (-11.3)
C.D (P =0.05)			3.39
C.D (P =0.01)			4.90

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

control of disease which ranged between 50-66% and reduction of lesion numbers by 28-52%. Reduction of disease mostly by reducing lesion numbers was recorded in cycloheximide and reducing number and size of lesions by sodium malonate, sodium azide and sodium iodoacetate. The effect of the chemicals became reduced with age in cycloheximide, sodium malonate and sodium azide showing 16-39% reduction of disease take place by reduction of lesion size and in others by reduction of both size and number of lesions. The effect of leaf diffusates of 14 days old plant in different treatments showed 43-68% 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 *Helminthosporium sativum*. All the chemicals showed appreciable control of disease ranged between 46-57% and reduction of lesion numbers by 35-43%. The reduction of disease caused by reduction of lesion size as well as lesion numbers.

DISCUSSION

All the five metabolic inhibitors used for wet seed treatment provide 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 chemicals at their effective concentration caused significant reduction in the number of lesions, particularly at 3-weeks old plants. But in some of the tretments at their effective concentration, showed the reduction of mean lesion size implying appreciable inhibition of lesion enlargement at this stage. Similar effects were also pronounced in older plants i.e. at 5-weeks old plants.

Moderate 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 much more toxicity under similar conditions. The greater amount of post-infectionally developed fungitoxicity in the treated plants, even 5 - weeks after seed tretment, appeared to be more significant than the initial development of toxicity in them. The above result may cause from the interaction between seed treatment and infection, mediated possible through some alteration in host metabolism. Some of the chemicals are also effective to induce resistance at significant level against natural infection in field condition.

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		
		14days	24days	24days			38days	38days		
Water (Control)		28.3	66.0	58.7 (-11.1)	32.9	8.6	61.5	54.2 (-11.8)	39.8	9.4
Cycloheximide	10^{-4}	8.9 (-68.0)	42.8 (-35.2)	32.1 (-51.4)	15.8 (-52.0)	2.9 (-66.3)	52.2 (-15.1)	36.3 (-41.0)	26.6 (-32.2)	6.0 (-38.8)
Sodium malonate	10^{-4}	15.9 (-43.6)	51.3 (-22.3)	36.0 (-45.5)	21.8 (-33.7)	3.7 (-57.0)	55.6 (-9.6)	38.8 (-36.9)	28.0 (-29.6)	6.8 (-30.6)
Sodium azide	10^{-4}	14.5 (-48.8)	53.2 (-21.2)	40.1 (-39.2)	23.7 (-28.0)	4.3 (-50.0)	55.2 (-10.2)	41.1 (-33.2)	39.5 (-0.8)	8.2 (-16.3)
Sodium iodoacetate	10^{-3}	10.0 (-64.5)	52.5 (-20.9)	39.2 (-40.6)	22.1 (-32.8)	4.0 (-53.5)	56.1 (-8.8)	50.8 (-17.4)	30.7 (-22.9)	9.3 (-5.1)
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=0.01)= 10.63 (24 days) 9.20 (38days).
* 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.

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

Chemical	Conc.	Mean no. of spots/plant.*	Mean disease index/plant.*
Water (Control)		14.4	5.2
Cycloheximide	10_{-4}	8.8 (-38.9)	2.0 (-57.7)
Sodium malonate	10_{-4}	9.3 (-35.4)	2.7 (-48.1)
Sodium azide	10_{-4}	8.1 (-43.8)	2.8 (-46.2)
Sodlium iodoacetate	10^{-3}	8.3 (-42.4)	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.

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