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Induction of phytoalexin in Ricebean *(Vigna umbellata)* by abiotic and biotic elicitors and its modulation by elevated temperature

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Several chemical compounds were tested for their ability to induce phytoalexin production in epicotyls of ricebean plants. Silver nitrate, cadmium sulphate, cupric chloride and cobalt chloride were effective inducers among inorganic compounds. Among organic compounds thioglycollic acid, 2, 4, 5-trichloroacetic acid and chitosan induced higher amounts of phytoalexin. The production of phytoalexin in ricebean plant was influenced by posttreatment incubation temperature. At elevated temperature, the production was much inhibited in treatments with abiotic elicitors, parallel to that following challenge inoculation by a fungus non-pathogenic to the plant. However, the induction by chitosan, a biotic elicitor was not supressed even at higher temperature. The practical importance of the results have been discussed.

Key Words : Ricebean, Phytoalexin, Elicitor, Temperature effect, Phytoalexin production

INTRODUCTION

Ricebean *(Vigna umbellata)* is known to produce phytoalexin phaseollin following infection by non-pathogen or pathogen of the plant (Datta *et al.*, 1986). Phytoalexin production has been found to be inhibited when inoculated plants are exposed to elevated temperature (Kundu, 1989). The present work was undertaken to test a wide range of abiotic and biotic elicitors for their ability to elicit phytoalexin production in tissues of ricebean and to examine whether chemical induction of phytoalexin synthesis is also repressed at elevated temperature.

MATERIALS AND METHODS

Epicotyls of seven-day old ricebean plants (cv. K—1) grown in dark were used as the plant material. *Helminthosporium oryzae*, a non-pathogen of ricebean was used to inoculate plant tissues and as the test organism for spore germination bioassay. The methods of phytoalexin induction and bioassay were similar to those described by Datta *et al*. (1986). Ricebean epicotyls were challanged with a condial suspension of *H. oryzae* or treated with dilute solutions of chemical compounds in sterile petridishes and incubated at 25°C for 24 hours. Epicotyls treated with distilled water served as controls. The bathing solutions of the of the control retrieved after incubation were considered as exudates and those with conidial suspension or chemical treatment as diffusates. After centrifugation, the resulting fluids were tested for fungitoxicity by slide germination bioassay technique (APS, 1943). Aliquot (100 μl) of exudate or diffusate was mixed with 2 μl of dense conidial suspension of *H. oryzae* and percentage germination of conidia and germ tube growth in different treatment regims were recorded. Extracts of phytoalexin from epicotyl tissues following inoculation or chemical treatment were made as described by Datta *et al* (1986).

RESULTS

Biotic and abiotic induction of phytoalexin

For induction of phytoalexin by fungal inoculum, epicotyl segments (5-6 cms) of 7-day old ricebean seedlings were challanged with dense conidial suspension of *H. oryzae* in sterilized petridishes, the volume of which was adjusted so as to submerge half of the girth of epicotyl segments. For chemical induction, aqueous solutions of sublethal concentration of test compounds as determined by a previous spore germination bioassay were prepared The epicotyl segments were treated in the same way as with fungal inoculation. For convenience, the treatments were done in two seperate lots : (i) with inorganic compounds and (ii) with organic compounds. Epicotyls taken in distilled water incubated in identical condition for 24 hours. Funitoxicity of diffusates from inoculated and chemically treated epicotyls were compared with exudates using conidia of *H. oryzae* as bioassy organism. The production of fungitoxic compounds by the test solutions was expressed in terms of inhibition of conidial germination and germ tube growth Results (Table 1) shown that diffusates from fungal inoculation were significantly fungitoxic as also those from some of treatments with chemicals while the exudates were almost non-toxic to bioassay organism. The results further show that silver nitrate, cadmium sulphate, cupric chloride and cobalt chloride are capable of inducing appreciable amounts of phytoalexin. Rest of the compounds were ineffective in stimulating the production of antifungal compounds. It is of interest to note that diffusates exerted greater

Table 1. Induction of phytoalexin in ricebean epicotyls following inoculation and treatment with inorganic compounds

Treatment	Bioassayed on conidia of *H. oryzae*	
	Germination (%)	Germ tube growth (μ)
Water (control)	90.0	456.0
Exudate[a]	85 (5.0)[c]	406 (10.9)
Diffusate[b]	18 (80)	46 (89.2)
Diffusates of[d]		
Silver nitrate (10^{-6}M)[e]	68 (24)	97 (78)
Cadmium sulphate (10^{-5}M)	80 (11)	76 (83)
Cupric chloride (10^{-6}M)	73 (19)	116 (74)
Cupric sulphate (10^{-6}M)	82 (8)	449 (1.5)
Cobalt chloride (10^{-6}M)	87 (3)	103 (77)
Mercuric nitrate (10^{-6}M)	88 (2)	432 (5)
Nickel chloride (10^{-5}M)	85 (5)	421 (8)

a Ambient solution from healthy epicotyls incubated in distilled water
b Ambient solution from *H. oryzae* inoculated epicotyls
c Figures in parentheses represent percentage of inhibition with respect to water control.
d Ambient solution harvested from epicotyls treated with indicated chemicals
e Concentrations of chemicals in parentheses indicate the doses of compounds non-lethal to the test organism

inhibitory effect on germ tube growth than on spore germination. In this respect, diffusate from cadmium sulphate treatment was most effective followed by that from silver nitrate.

In a seperate experiment, five organic compounds were tested for their ability to induce phytoalexin production. Results (Table 2) showed that chitosan, thioglycollic acid and 2, 4, 5—trichloroacetic acid (2, 4, 5-T) were able to stimulate phytoalexin production. Among these, diffusates from chitosan treatment showed fungitoxicity very near to that shown by fungal inoculation. Here also,

Table 2. Formation of phytoalexin in epicotyls of ricebean following treatment with some organic compounds

Chemical compounds	Conc. used	Conc. of diffusate tested	Bioassay of diffusates against conidia of *H. oryzae*	
			Germination (%)[a]	Germ tube growth (μ)[b]
Water (control)	—	—	92	443
Thioglycollic Acid	10^{-4}M	X[c]	73 (20)[d]	121 (72)
		X/2	90 (2)	286 (35)
2, 4, 5-Trichlorocetic Acid	10^{-4}M	X	76 (17)	133 (70)
		X/2	82 (10)	210 (52)
p-chloromercuric benzoate	10^{-5}M	X	90 (2)	437 (1.5)
		X/2	89 (3)	443 (0)
Cycloheximide	10^{-5}M	X	90 (2)	442 (0.2)
		X/2	92 (0)	440 (0.6)
Chitosan	300 ppm	X	32 (65)	90 (79)
		X/2	69 (25)	256 (42)

[a] Calculated on the basis of 200 observations

[b] Results represent mean of 50 observations

[c] Undiluted ambient solution from epicotyls treated with chemicals considered as X

[d] Figures in parentheses denote percent reductions with respect to control

it was observed that phytoalexin (s) were more inhibitory to germ tube growth of bioassay organism. Cycloheximide and p-chloromercuric benzoate could not induce any detectable amounts of antifungal compounds in diffusates.

Effect of temperature on phytoalexin production

Epicotyl segments were challanged with conidial suspension of *H. oryzae* or solution of chemical compounds as described earlier. Epicotyls taken in distilled water served as control. Following the treatment, epicotyls taken in petridishes were incubated at three different temperature conditions viz. 20° 28° and 37°C for 24 hours. Thereafter the resulting exudates and diffusates were collected and tested for fungitoxicity using conidia of *H. oryzae*. For comparison of abiotic induction of phytoalexin (s) with that following fungal inoculation, epicotyls challanged with spore suspension were also incubated at temperature conditions similar to those of chemical induction. Results (Table 3) showed that epicotyls incubated at lower range of temperature (20°C) produced phytoalexin in significant amount following both fungal inoculation and chemical treatment whereas control epicotyls produced very small amounts. With further increase in incubating temperature, the degree of inhibitory effect of diffusates from treatment with

Table 3 Effect of incubation temperature on production of antifungal compounds by ricebean epicotyls following inoculation with a non-pathogen and treatment with biotic and abiotic elicitors

Inducer used	Incubating temp. (°C)	Percent inhibition[a] of conidial germination and germ tube growth of bioassay fungus, *H. oryzae*	
		Germination	Germ tube growth
Water (Exudate)	20	9	4
	28	7	2
	37	4	0.9
Conidia of *H. oryzae* (Diffusate)	20	21	85
	28	11	64
	37	8	34
Silver nitrate (10^{-6}M)	20	52	68
	28	42	53
	37	8	17
Cadmium sulphate (10^{-5}M)	20	41	77
	28	36	52
	37	15	17
Chitosan (300 ppm)	20	64	84
	28	45	80
	37	38	75

[a] For bioassay of fungitoxicity conidia of *H. oryzae* were used. Percentage of inhibition was calculated with respect to values in water control, wherein the germination and average length of germ tubes were 90% and 445μ respectively.

Table 4. Effect of post treatment incubation temperature on endogenous production of phytoalexin(s) in ricebean epicotyls following treatment with abiotic and biotic elicitors

Elicitors used and concentration	Incubation temp. (°C)	Dilution of test solution	Bioassay of treatment solution[a] against conidia of *H. oryzae*	
			Conidial germination (%)[b]	Germ tube length (μ)
Water (control)			93	469
Silver nitrate (10^{-6}M)	20	X[c]	63 (32)[d]	39 (91)
		X/2	80 (14)	350 (25)
	28	X	73 (21)	65 (86)
		X/2	82 (12)	210 (55)
	37	X	90 (3)	240 (48)
		X/2	90 (3)	381 (19)
Cadmium sulphate (10^{-5}M)	20	X	40 (57)	43 (91)
		X/2	61 (34)	156 (67)
	28	X	52 (44)	242 (48)
		X/2	58 (37)	328 (30)
	37	X	58 (37)	305 (34)
		X/2	84 (9)	456 (3)
Chitosan (300 ppm)	20	X	5 (95)	8 (92)
		X/2	28 (70)	48 (89)
	28	X	38 (59)	27 (94)
		X/2	62 (33)	202 (60)
	37	X	48 (48)	38 (91)
		X/2	68 (27)	212 (54)

[a] Epicotyls following abiotic and biotic induction were extracted with ethyl acetate which was flash evaporated and residues dissolved in small volume of water for bioassay

[b] Slide germination bioassay done using conidia of *H. oryzae*

[c] 'X' represents undiluted test solution

[d] Figures in parentheses indicate percent inhibition with respect to water control, based on 100 observations for germination and 50 for germ tube length

silver nitrate and cadmium sulphate gradually decreased as also in the treatment with silver nitrate and cadmium sulphate gradually decreased as also in the treatment with fungal inoculation. Apparently the phytoalexin production diminished at higher temperature. Diffusates collected from treatments at 37°C were least inhibitory.

It was of interest to note that at elevated temperature (37°C), phytoalexin induction by silver nitrate and cadmium sulphate was severely depressed as the diffusates supported better spore germination and good germ tube growth. In contrast to this, diffusates from chitosan treatment at the corresponding temperature showed much higher antifungal activity.

Endogenous phytoalexin level following abiotic elicitation

Since in the previous experiment it was observed that phytoalexin production was affected to a large extent by the ambient temperature condition, this experiment was done to test the effect of temperature condition on the endogenous level of antifungal compounds in ricebean tissues following induction by three selected chemical compounds and incubation at different temperature conditions. After 24 hours, tissues were extracted with 80% alcohol and then with ethylacetate after evaporating the alcohol. The extracts were bioassayed for fungitoxicity.

Results (Table 4) showed that the extracts of epicotyls of the control tissue were not inhibitory to the bioassay organism while those prepared from epicotyl incubated at lower range of temperature(20°C) appeared to be inhibitory in all treatments. It is evident from the results that at elevated temperature of 37°C, the endogenous phytoalexin production by the abiotic elicitors, silver nitrate and cadmium sulphate was diminished remarkably while it was not inhibited to any appreciable level in treatment with chitosan, the biotic elicitor. The extract of tissues incubated with chitosan at elevated temperature caused strong inhibition of germ tube length and substantial supression of conidial germination.

DISCUSSION

The present investigation confirms the previous reports (Datta *et al.*, 1986; Kundu, 1989) that epicotyls of ricebean plant are capable of producing significant amount of phytoalexin-like compounds following invasion by *H. oryzae*, a fungus, non-pathogenic to the plant. Further, it has been shown that several other biotic elicitors also could elicit the production when applied in a very low concentration. Thus the induction of phytoalexin (s) in ricebean tissue is in conformity with reports of earlier authors, claiming the production of such compounds in other plant species (Perrin and Cruickshank, 1965; Hadwiger, 1972; Hadwiger and Beckman, 1980 and Cruichshank, 1980). The results further indicated that chemical induction of phytoalexin in this plant is substantially repressed similar to that following fungal inoculation and incubation at higher temperature, except in case of chitosan. Examples of such high temperature-induced inhibition of phytoalexin synthesis are also found in the literature (Cruickshank, 1963 ; Classen and Ward, 1985). Little or no production of phytoalexin when inoculated tissues were incubated at 35-37 has been attributed to destruction of a key enzyme involved in its synthesis (Kundu, 1989) but in the light of results reported here, it is not possible to say whether the same phenomenon also occurs in abiotic elicitation process.

It was of interest to note that endogenous production of phytoalexins or their release into ambient solution following chitosan treatment and incubation at elevated temperature was not much inhibited. This means that tissues retain phytoalexin synthesizing ability even at higher temperature. This observation has some practical importance. At higher temperature, disease resistance of ricebean plants decreases and plants become susceptible, particularly to *Macrophomina phaseolina*, a very common fungal pathogen found in most tropical soils. Since phytoalexins have been thought to play a vital role in protecting plants from potential pathogen (Van Etten and Pueppke, 1976) as a factor in the multicomponent defence system of plants, our results indicate that chitosan treatment may protect ricebean plants from infection by *M. phaseolina* at elevated temperature conditions under which these plants are grown in greenhouse or in open fields.

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