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Increased Susceptibility of Mungbean to *Macrophomina phaseolina* At Low Temperature

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The effect of ambient temperatures on the mungbean seedling damping off was studied, Cold temperature (25°C) favoured the incidence of damping off of the potted seedlings inoculated with maizemeal-sand inoculum of *M. phaseolina.* Varying ambient temperature conditions had little or no effect on the percentage germination and root and shoot growth of mungbean. Challange inoculation with propagule suspension of *H. oryzae* (non-pathogen) or *M. phaseolina* (pathogen) induced phytoalexin like compounds in the 7-day-old hypocotyls of mungbean. Post inoculation low temperature treatment of the hypocotyls resulted in significant decrease of phytoalexin potential. The low level of endogenous and effluxed phenol contents of hypocotyl at low temperature as compared to those of higher temperature, clearly indicated a low temperature induced reduction of phenolics including the phytoalexins and consequent breakdown of resistance. Low temperature caused decrease in the soluble sugar contents but had variable effect on the proline content of the tissues following infections.

Key words : Mungbean *(Vigna radiata* var. *aureus)* ; *Macrophomina phaseolina* ; Low temperature predisposition ; Phytoalexin.

INTRODUCTION

Mungbean or green gram or golden gram (*Phaseolus aureus* Roxb.= *Vigna radiata* var. *aureus*) is one of the most important pulses of India and other tropical countries. Mungbean is cultivated mainly as a *Kharif* crop in most of the states of India, it is also grown as *Rabi* crop in southern India where the winter is quite mild (Kachroo, 1970).

There are several reports of cold temperature induced predisposition of plants to microbiol invasion in the literature (Yarwood, 1976 ; Colhoun 1979 ; Bell, 1981). Cold induced susceptibility to infection has been reported for *Alternaria* on pepper (Mc Colloch, 1962), *Botrytis* on grapes (Ciccarone, 1959),

Pyricularia on rice (Manibhusanrao and Dey, 1972) and *Rhizoctonia* on beans (Schulz and Bateman, 1969). Mungbean is susceptible to *M. phaseolina* root rot and the disease becomes extremely severe when grown in Rabi season or in winter (Kachroo, 1970 ; Singh *et al.*, 1970).

Elevated temperature induced increased susceptibility of certain host-parasite systems have been reported to be due to inhibition of phytoalexin synthesizing potential of the host (Cruickshank 1963 ; Chamberlain and Gardemann, 1966 ; Bailey *et al.*, 1980 ; Keen *et al.*, 1981). The biochemical basis of cold temperature induced susceptibility is poorly understood.

Severe infection of mungbean by *M. phaseolina* during winter season indicates a possible break down of defence mechanism at cold temperatures. Mungbean is known to produce at least three phytoalexins such as kievitone, phaseollidin, and phaseollin in the hypocotyls following challange inoculation (Ingham, 1982). Little or no information is available on the change in the phytoalexin level of mungbean plants exposed to different temperature stress conditions.
The objective of this investigation was to study the mechanism of cold temperature induced susceptibility of mungbean to *M. phaseolina* infection.

MATERIALS AND METHODS

Mungbean (*Vigna radiata* var. *aureus*) seeds were obtained from local market. Seedlings were grown in plastic trays lined with blotting paper and 7-day-old seedlings were used for most experiments. For temperature effects on germination and growth, seeds were sown as above and maintained at 25°, 30° or 35°C temperature controlled cabinets. The percentage germination and growth of root and shoots of the seedlings were recorded at 24 hrs. intervals.

Virulent cultures of *Helminthosporium oryzae* and *Macrophomina phaseolina* were obtained from the stock culture collection of the Plant Pathology Laboratory of Kalyani University. The organisms were maintained on Potato-dextrose-agar (PDA) slopes with monthly subculturing. Both the organisms produced abundant propagules on PDA after 10 to 15 days incubation at 28°C.

For determination of damping off mungbean seedlings were grown in plastic pots containing garden soil and maizemeal inoculum (maizemeal inoculum was prepared by mixing 10 g of maizemeal with 90 g of sand in a 250 ml Erlenmeyer flask). The mixture was sterilized by autoclaving and inoculated with *M. phaseolina* and grown for 21 days at 30°C. Entire content of the flask was considered as 100 g inoculum (in the ratio of soil 9 : 1 inoculum). Pots were sown with seeds and watered on alternate days. Seeds grown on pots containing only soil served as controls Inoculated and control pots were incubated at 25°, 30° or

37°C in temperature controlled cabinets. Percentage of post emergence damping off was calculated by recording the number of seedlings showing root rot or wilting against emergence in control pots.

For induction of phytoalexin, sections of mungbean hypocotyls of 7-day-old seedling (10 g) were placed as a monolayer in the bottom halves of petridishes (11 cm diam.) and flooded with 15 ml water, conidial suspension of *H. oryzae*, or sclerotial suspension of *M. phaseolina* (10^6 conidia or sclerotia per ml), covered with the lids and incubated at 25°, 30° or 37°C in temperature controlled cabinets separately. After 24 hrs. the treatment fluids were harvested, centrifuged (5000xg for 10 min) and the fungitoxic activities of the supernatant solutions were bioassayed. The fungitoxicity of exudates and diffusates obtained by different treatments were assayed by slide germination bioassay technique using *H. oryzae* as bioassay fungus. Percentage germination and germ tube growth after 24 hrs. of incubation were determined. Carbohydrate, phenol and proline contents of the tissues and ambient fluids of different treatments were deternined by the method of Morris (1948), Spies (1955) and Bates *et al.* (1973) respectively.

RESULTS AND DISCUSSION

Potted seedlings of mungbean with or without maizemeal-sand inoculum of *M. phaseolina* were incubated at different temperatures. The disease severity was calculated based on precentage of postemergence. Results (Table 1) indicated that control mungbean seedlings grew well at all the test temperatures and did not exhibit any post-emergence seedling mortality. Inoculated seedlings, however, showed low mortality at 30° or 37°C, but high mortality (77%) at low temperatures. The data suggest that *M. phaseolina* induced damping off of mungbean

Table 1 : Effect of ambient temperature on the incidence of damping off of potted seedlings of mungbean inoculated with maizemeal-sand inoculum of *Macrophomina phaseolina*.

Incubation temperature (°C)	Percentage of post-emergence damping off[a] of seedings	
	Control[b]	Inoculated[c]
25	0	77.3
30	0	6.9
37	0	4.5

a Seedlings after emergence showed root rot and consequent wilting.
b Garden soil without any inoculum of *Mecrophomina phaseolina*.
c Soils were inoculated with maizemeal-sand inoculum of *M. phaseolina*.

seedlings is favoured at low temperatures. Similar results of cold temperature induced increased disease severity has been reported previously by many authors using diverse host-parasite systems (Ciccarone 1959 ; Mc Colloch, 1962 ; Schulz and Bateman 1969 ; Manibhusanrao and Dey, 1972).

Germination of seeds, length of root and shoot of seedlings exposed to different temperature conditions were determined. Result (Table 2) showed that percentage germination of mungbean seeds was 100% irrespective of the ambient temperature conditions. The high temperature regimes (35° and 37°C), however, were somewhat inhibitory to root and shoot growth of mungbean seedlings. Apparently, at lower temperature, the plants vigour was not significantly affected.

Table 2 : Effect of temperature on the germination of seeds and seedling root and shoot growth of mungbean.

Incubation temperature (°C)	Percent[a] seeds germinated	Length (cm) of root or shoot after days (D) of incubation			
		Root[b]		Shoot[b]	
		3D	5D	3D	5D
25	100	6.7	9.9	6.6	16.3
30	100	6.2	6.5	6.8	13.9
35	100	7.1	7.2	6.6	11.3
37	100	7.1	7.4	5.5	10.8

a Germination of seeds in Petridishes lined with moist filter paper was determined.

b Seedling root and shoot growth in Petridishes was measured after indicated days of intervals.

Since the seedling damping off was more severe at low temperatures, the possibility of relative decrease in phytoalexin produced by the hypocotyls exposed to inoculation and low temperature conditions were investigated. Hypocotyl sections following challange inoculation were incubated at low and high temperature conditions and the fungitoxity of the diffusates were bioassayed. Results (Table 3) showed that the diffusates of *M. phaseolina* obtained by incubating the hypocotyls at 37°C were strongly inhibitory to germination (96%) and germ tube growth (85%) of *H. oryzae*. In contrast, the diffusates obtained by incubation at 25°C was significantly less inhibitory to bioassay fungus, suggesting that hypocotyls of mungbean produced less phytoalexins at lower temperatures. Diffusates of *H. oryzae* although exhibited similar trend of decrease of phytoalexin production at lower temperature, exhibited somewhat variable fungitoxicity to the bioassay fungus.

The results suggest decreased production of phytoalexin at low temperature with consequent break down of resistance of plants to infection. Similar low temperature induced reduction in phytoalexin content with consequent increased severity

Table 3 : Effect of temperature on the the induction of phytoalexin like compounds in mungbean hypocotyl sections by pathogen and non-pathogen of the host as measured by the fungitoxicity of exudates and diffusates using slide germination bioassay.

Test[a] solutions	Incubation[b] temperature (°C)	Spore germination bioassay using conidia of *H. oryzæ*			
		Germination[c] (%)	Inhibition (%)	Germ tube[d] growth (μ)	Germ tube inhibition (%)
Diffusate from *H. oryzæ*	25	46.0[e]	54 0	109.6	66.1
	30	41.7	58.3	101.3	68.6
	37	38.9	61.1	99.6	69.2
Diffusate from *M. pheseolina*	25	63 8	36.2	137.7	57.3
	30	45.1	54.9	117.8	63.5
	37	19.4	88.6	91.3	71.7

[a] Percent germination and garm tube growth in water was considered as control. Exudate was obtained by flooding hypocotyl sections in water. Diffusate from non-pathogen or pathogen were obtained similarly with spore or sclerotial suspension respectively.

[b] Hypocotyl sections following challange inoculation were incubated in different temperature controlled cabinets.

[c] Percent germination was calculated based on at least 200 spores.

[d] Growth of germ tube was measured using micrometers.

[e] Test solutions were diluted 50% with water to give X/2 dilution.

of disease has been reported by other workers using different host-parasite systems (Bell and Parsley 1969 ; Bell, 1981).

The low level of endogenous and effluxed phenol contents of mungbean hypocotyls at low temperature but increase in levels of phenols both inside the tissues and in the ambient fluid with increase in temperature (Table 4) further support the idea that the defence mechanism of the plant involving phytoalexins, which

Table 4 : Comparison of the effects of temperature on the endogenous and effluxed carbohydrate, proline and phenol contents of mungbean hypocotyl sections following challange inoculation and incubation at different temperatures.

Hypocotyls treated with	Incubation temperature (°C)	Contents of metabolites equivalent to					
		Anthrone positive sugar		Proline		Chlorogenic acid	
		Tissue (mg/g)	Ambient fluid (mg/g)	Tissue (μ mole/g)	Ambient fluid (μ mole/g)	Tissue (μg/g)	Ambient fluid(μg/g)
Water	25	9.6	36.0	6.3	0	280	108
	30	8 4	45.0	9.3	0	280	330
	37	4.8	75.0	10.4	4.8	320	394
Sclerotial suspention of *M. phaseolina*	25	10.0	192.5	9.3	9.6	200	156
	30	8.6	2o5 0	10.4	9.6	320	216
	37	5.2	285.0	10.4	9.6	360	300

are phenol derivatives, decreased at low temperature causing break down of resistance. Carbohydrate contents of control and inoculated tissues were more at lower temperature, but decreased with increase in temperature. In contrast, carbohydrate contents of diffusates from the pathogen inoculated hypocotyls increased with increase in temperature, but remained unchanged in the diffusates of the hypocotyls inoculated with the non-pathogen. In general, more carbohydrates leaked out into the ambient solution following inoculation as compared to water controls. The exact explanation of this need further investigation. Proline is known to be a stress metabolite produced by different plants in response to environmental and pathological stresses. The control tissues produced more proline with increases in temperature and little or no proline is excreted out in exudate at lower temperature, but about 4.8μg/ml is exuded at 37°C. Non-pathogen inoculated hypocotyls showed similar trends. Hypocotyls inoculated with pathogen, however, showed high level of proline irrespective of the temperature conditions (Table 4). In several other host-parasite systems increased level of proline following infection has been reported and it is assumed that proline accumulation may provide some means of protection against stress imposed by temperature or infection (Stewart and Carher, 1990). The role of proline in the production of hydroxyproline rich glycoproteins which are important in host defence has been suggested (Bolwoll *et al* , 1985). The exact relationship between proline, temperature stress and infection, however, is poorly understood at present and needs investigations.

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