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Role of Hydrolytic Enzymes in Seedling Blight of Jute Incited by *Macrophomina Phaseolina*

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Experiments conducted on the role of pectinolytic and cellulolytic enzymes in pathogenesis of seedling blight of jute incited by *Macrophomina phaseolina* clearly demonstrated positive involvement of both these enzymes. Both these enzymes were found to be produced by the pathogen constitutively in Czapek Dox medium and inductive response to enzyme production in the substrate in which pectin and carboxymethyl cellulose were introduced was limited. Both these enzymes could be extracted from the infected and apparently healthy regions in close proximity to infected ones in different stages of disease development. Fourteen days old seedlings of jute kept in enzyme solution at 21°C for 48 hours showed distinct lesions in the cotyledonary region.

Brown (1936) in his classical works pointed out the role of pectinolytic enzymes in pathogenesis particularly in facultative parasites producing soft rot.

Uritani and Stahmann (1961) in discussing the role of pectinolytic enzymesin pathogenesis in dry rot of sweet potato ascribed three possible functions, namely (a) degradation of pectic substances to provide nutrients and energy for growth and penetration of pathogen, (b) maceration of host cells to allow the mycelium of the pathogen to penetrate the cells killed due to the action of the enzymes and absorb nutrients and (c) decomposition of pectic substances with interference in active metabolism related to instant necrosis or supersensitive reaction.

Macrophomina phaseolina (Tassi.) Goid is a typical facultative parasite with wide host range. Chan and Sackston (1970 a, b, 1971, 1972) pointed out that *M. phaseolina* could produce both pectinolytic and cellulolytic enzymes both in virto and in vivo resulting in degradation of cell wall of sunflower stem. Rai and Srivatsava (1975) showed that *M. phaseolina* incitant of stem and root of *Brassica juncea* secreted endo-polygalacturonase and cellulase in virto and in vivo PG-activity was found to have a direct correlation with virulence of various isolates.

In Jute (*Corchorus capsularis* and *C. olitorius*), nature of major symptoms, namely seedling blight and damping off in early stages and rotting of fibres which are primarily of cellulose resulting in shredding in the adult stages strongly suggests involvement of pectinolytic and cellulolytic enzymes in pathogenesis in the disease incited

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by *M. phaseolina* (Chatterjee and Basu, 1960). Accordingly studies were taken up to find out the role of these enzymes in seedling blight of jute.

MATERIALS AND METHODS

For preparation of enzyme extracts, isolate S_8 (of plant Pathology Laboratory, BCKVV) of *M. phaseolina* was grown in Czapek Dox Broth in 250 ml conical flasks. After eight days of incubation at 27±1°C, mycelial mats were separated by filtering through Buchner funnel using Whatman No. 1 filter paper. The mats with the filter paper were dried at 70° in an incubator. Filtrate was kept aside. Dried fungal mats were chilled at 0°C in a mortar and later crushed with a pestle. This operation was twice repeated. Distilled water was added to the crushed mycelial mats in the ratio of 1 : 10. Inductive nature of the enzymes was studied by incorporating 1.2 and 2.4 per cent pectin and carboxymethyl cellulose in the medium for pectinolytic and cellulolytic induction respectively. The fungus was grown for 6, 8 and 10 days at 27 ± 1°C in an incubator.

Twenty day old seedlings of jute (*Corchorus capsularis* L.) were inoculated with four day old cultures of four different isolates of *M. phaseolina*. For purposes of investigation, infected hosts were divided into three regions, namely (a) root region, (b) infected region (c) apparently healthy region (one cm above the infected region). Course of infection was divided into three stages namely, Stage I-characterised by presence of brown water soaked lesions on the cotyledonary region, Stage II-enlargement of black lesions on the stem and drooping of the host and Stage III complete collapse of the host. Enzyme extraction from the infected host tissue was done by the same procedure as above. Extracts were centrifuged at 3000 rpm for 10 minutes at 5°C, Pectinolytic and cellulolytic enzymes were assayed viscosimetrically using 1.2 per cent sodium polypectate and 1.2 per cent carboxymethyl cellulose as substrates respectively buffered with 0.1 M citrate buffer at pH 6.5.

The reaction mixture consisting 3 ml of substrate, 2 ml of distilled water and 1 ml of enzyme preparation in 10 ml Ostwald Viscosimeter tube was incubated at 30° C for twelve hours. Loss of viscosity was recorded in Ostwald Viscosimeter by a 1/10 second time recorder. Loss of viscosity was calculated by the following formula.

$$\frac{No - Nt}{No - Nw} \times K$$

No - viscosity at '0' time,
Nt - viscosity at time 't',
Nw - viscosity of water, K = 100. Three replications were used in all cases (Colowick and Kaplan, 1955).

RESULTS AND DISCUSSION

Data on assay of activity of pectinolytic and cellulolytic enzymes are presented in Table I, each figure in the table representing an average of three.

From the data presented in Table I, it is evident that *M. phaseolina* secreted both pectinolytic and cellulolytic

TABLE I. Activity of constitutive and inductive pectinolytic and cellulolytic enzymes of *Macrophomina phaseolina*

Nature of enzymes		Per cent reduction in viscosity Days of incubation					
		6 days		8 days		10 days	
		(a)	(b)	(a)	(b)	(a)	(b
Constitutive		70	79	80	26	91	46
Inductive	(i)	64	70	100	100	100	24
	(ii)	65	70	100	99	100	96

a) Pectinolytic enzymes: (b) cellulolytic enzymes

i) 1.2% pectin and 1.2% carboxymethyl cellulose in the medium (W/V) for pectinolytic and cellulolytic enzyme study respectively.

ii) 2.4% pectin and 2.4% carboxymethyl cellulose in the medium (W/V) for pectinolytic and cellulolytic enzyme study respectively.

enzymes. In case of cellulolytic enzymes, a sharp decline in activity was noticed with age, though a small rise occurred at 10 days of incubation after a sharp fall at eignt days of incubation. Response to substrate in the production of inductive pectinolytic enzymes was not very marked. At six days of incubation, no difference in activity between constitutive and inductive enzymes could be detected and in later stages, increase in activity due to addition of pectin in the medium was not very high. Increase in concentration of substrate did not show any response.

In case of cellulolytic enzymes, response to substrate in inductive production was more marked. At six day stage however, increase in activity due to addition of carboxymethyl cellulose was noticed. At eight day stage, while the activity of constitutive enzyme decreased sharply, the same of inductive enzyme increased greatly. This rise in activity was maintained at 10 day stage only in the higher concentration of the substrate in the medium in the lower concentration activity showed sharp fall.

Data on the activity of pectinolytic and cellulolytic enzymes extracted from the infected host tissues of jute *Corchorus capsularis*, Variety D 154) were recorded in two ways, namely (i) in different regions pooling different stages of disease development, and (ii) at different stages of disease development pooling different regions of infection. Results based on average of three replicates are presented in Tables II and III.

From the data presented in Tables II and III, it is evident that both

TABLE II. Activity of pectinolytic and cellulolytic enzymes extracted from the different regions of infected host.

Isolate	Enzymes	Percentage reduction in viscosity		
		Root region	Infected region	Apparently healthy region
S_1	Pectinolytic	33	33	0
	Cellulolytic	48	41	62
S_2	Pectinolytic	40	36	37
	Cellulolytic	45	40	46
S_7	Pectinolytic	24	31	28
	Cellulolytic	40	40	46
S_8	Pectinolytic	33	67	43
	Cellulolytic	45	43	64

TABLE III Activity of pectinolytic and cellulolytic enzymes at different stages of disease development.

Isolate	Enzymes	Percent reduction in viscosity Stages of disease development		
		I	II	III
S_1	Pectinolytic	30	20	17
	Cellulolytic	54	50	47
S_2	Pectinolytic	54	16	40
	Cellulolytic	46	45	40
S_8	Pectinolytic	21	25	33
	Cellulolytic	44	42	41
S_7	Pectinolytic	50	47	80
	Cellulolytic	58	46	47

pectinolytic and cellulolytic enzymes were involved in pathogenesis. Both these enzymes were extracted from infected hosts at different stages of disease development as well as in different regions including apparently healthy regions.

In respect of cellulolytic enzymes, variations in activity were not observed in different stages of development, and in different regions excepting apparently healthy region from which a consistently greater activity was recorded. Pectinolytic enzymes showed variations in activity both in relation to regions as well as stages of development of the disease. In general greater activity of pactinolytic enzymes was noticed in the infected region and in the earlier and later stages of disease development. Differences among the isolates in respect of enzyme activity were not very marked. In general isolate S_8 showed greater activity particularly in respect of pactinolytic enzymes.

Fourteen days old seedlings of jute (*Corchorus capsularis* Variety D 154) were introduced in test tubes containing 5 ml of cell free enzyme preparations of *M. phaseolina*. Seedlings were held in position with roots immersed in solution preparation by sterile cotton plugs in the tube in each of which seedlings were kept and incubated at 21°C for 48 hours. Altogether ten tubes, and fifty seedlings were used. After the period of incubation, brown water soaked lesions wers notied on the cotyledonary regions of the seedlings. The symptoms observed were similar to those noticed in Stage I of the development of the disease. Seedlings kept in control sets (heat inactivated enzyme preparation and

Czapek Dox broth without the fungus) did not show any such symptoms. Microscopic observation of the damaged host tissue showed maceration and thinning of cell wall and loss of coherence of cells the typical symptoms of activity of pectinolytic enzymes as reported by Brown (1936), and Bateman (1963, 1964). These observations further confirm the role of pectinolytic and cellulolytic enzymes in pathogenesis of seedling blight of jute incited by *M. phaseolina*.

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