# Enzyme activity in banana fruits rotted by Botryodiplodia theobromae Pat.

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### Abstract

Peroxidase and polyphenol oxidase activities in fruits of two cultivars of banana, 'champa' and 'kanthali' rotted by *Botryodiplodia theobromae* Pat. was studied. The enzymes showed much higher activities in infected than that in uninfected tissues. Increase in peroxidase activity was evidently inhibited by cycloheximide. Polyphenol oxidase activity was also inhibited in presence of phenylthiourea and Na-diethyldithiocarbamate more strongly by the former. Increase in activities seemed to be due to increased sytheses of the enzymes. In an *in vitro* culture, the fungus exhibited some peroxidase but no polyphenoloxidase activity.

## INTRODUCTION

Increased peroxidase and polyphenoloxidase activities in host tissues infected by fungi have been reported by a number of workers (Matta and Dimond 1963; Hare 1966; Weber et al., 1967; Rubin et al. 1971). The increase of such enzyme activities are known to be due to activation of latent enzymes in the host or appearance of new isoenzymes. Increased activity of polyphenoloxidase has also been reported to be due to its production by the infecting fungi, Cochliobolus miyabianus (Oku 1960) and Puccinia graminis tritici (Farkas and Ledin-gham 1959). In the present study, activities of these two enzymes in banana fruits rotted by Botryodiplodia theobromae Pat. has been investigated.

## MATERIAL AND METHODS

The fungus was isolated from rotted banana fruits collected from the local market in Burdwan. The identification of the species was confirmed by I.A.R.I., New Delhi. Healthy banana fruits of 'champa' and 'kanthali' varieties commonly available in and around Burdwan, were ino-

culated with mycelial bits (5 mm  $\phi$ ) of the fungus by placing the inocula on the surface of the pulp below the skin and replacing the skin. The inoculated bananas were incubated at 30°C and 80% relative himudity. At intervals of 24 hrs. small bits of pulp tissues taken from the inoculation sites were studied for the enzymes. Tissues from fruits similarly injured but not inoculated with fungus and kept under identical conditions, served as control.

Extraction of peroxidase from infected and healthy tissues of banana was done following C h a t t o p a d h y a y and N a n d i (1976). Enzyme activity was recorded as change in absorbance per minute ( $\Delta$  A/min) at 430 nm immediately after the addition of  $\rm H_2O_2$  as substrate. Nonenzymatic oxidation was measured using extract which had been heated at  $100^{\circ}{\rm C}$  for 10 minutes. The activity was always zero indicating complete inactivation of the enzyme by heat treatment. Peroxidase activity of infected tissues in presence of cycloheximide was also tested. To study this, another set of experiments were done by placing infected cut tissues in a solution of cycloheximide (10  $\mu{\rm g/ml}$ ) or in water for control. The tissues were kept in humid condition to prevent desiccation. The tissues were then assayed for peroxidase after different periods of incubation.

Extraction of polyphenoloxidase from healthy and infected tissues was done following mainly Chattopadhyay and Nandi (1976). Since the activity of this enzyme remains adsorbed on or structurally associated with cell wall of tissue fragments (Palmer 1964), release of the enzyme into solution was accomplished by extraction with 1% detergent solution (non-ionic detergent, Tween 40) buffered at pH 7.0 with 0.1 M potassium phosphate. The extract was filtered through cheese cloth, centrifuged and the supernatant stored in ice until assayed. Activity of the enzyme was measured as the change in absorbance per minute (\Delta A/min) at 470 nm immediately after addition of catechol (2 mg/ ml) which initiated the reaction. Change in absorbance was also measured using extract which had been boiled at 100°C for 10 minutes. Activity in boiled extract was measured zero indicating complete inactivation of the enzyme by heat treatment. Polyphenoloxidase activity of tissues was also studied in presence of phenylthiourea or sodium diethyldithiocarbamate ( $6 \times 10^{-8}$  M) following Bateman (1962). Tissues, dipped in solutions of the compounds for different periods under humid condition, were analysed for the enzyme.

Enzymes present in mycelia of the fungus were extracted from thoroughly washed 6 days'old culture grown on liquid Czapec's medium in complete darkness. The activities of the enzymes were assayed following the same method used in case of infected tissues.

#### RESULTS

Healthy banana fruits showed some differences in both peroxidase and polyphenoloxidase activities between the two varieties which were slightly higher in 'champa' than that of 'kanthali'. Their activities remained more or less unchanged with prolongation of incubation.

In infected fruits, peroxidase activity showed a marked increase over that in control after 24 hours of incubation. The enzyme activity increased considerably in both varieties with increase in incubation time (Fig. 1A).

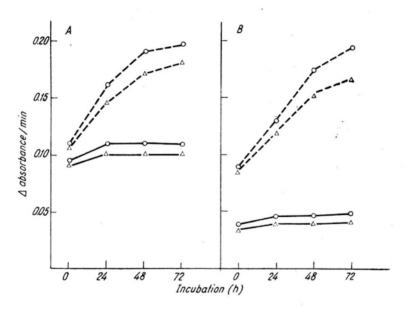


Fig. 1. Peroxidase (A) and polyphenoloxidase (B) activities in healthy and infected tissues of two cultivars of banana infected with Botryodiplodia theobromae after different incubation periods

continuous line - healthey; broken line - infected; cicrle - 'champa'; triangle - 'kanthali'

Polyphenoloxidase activity also showed an evident increase in both hosts after 24 hours (Fig. 1B). Activity of the enzyme showed further increase with time of incubation. The difference in the activity between the varieties, however, increased considerably with the length of the incubation period. After an initial increase, a slight depression in the activity of the enzyme was observed more evidently in 'kanthali' than the other variety.

In vitro culture, the peroxidase activity of the fungus was found to

Table 1

Peroxidase and polyphenoloxidase activities in healthy and infected (with  $B.\ the-$ obromae) tissues of two cultivars of banana fruits following treatments with different inhibitors for different incubation periods (expressed as  $\Delta$  A/min)

Tissue				Incubation period (hours)			
	Treatment	0		24		48	
		'Champa'	'Kanthali'	'Champa'	'Kanthali'	'Champa'	'Kanthali'
Peroxidase							
healthy	water	0.095	0.090	0.110	0.110	0.110	0.100
	cycloheximide	<u>·</u>	_	0.072	0.054	0.098	0.074
infected	water	0.110	0.110	0.160	0.140	0.185	0.165
	cycloheximide	-	_	0.110	0.100	0.120	0.115
Polyphenolox	idase						
healthy	water	0.040	0.030	0.045	0.040	0.045	0.040
	phenylthiourea Na-diethyl- dithiocarba-	-	_	0.022	0.008	0.030	0.009
	mate	-	_	0.035	0.025	0.065	0.060
infected	water	0.085	0.070	0.140	0.120	0.165	0.150
	phenylthiourea <b>Na-diethyl-</b>		_	0.020	0.010	0.030	0.020
	dithiocarba- mate		_	0.035	0.030	0.080	0.040

range between 0.03 and 0.19  $\Delta$  A/min but no activity of polyphenoloxidase was recorded.

Cycloheximide caused considerable decrease in peroxidase activities in both healthy and infected tissues as compared to their respective water treated controls (Table 1). Similarly, polyphenoloxidase activity was decreased in presence of both phenylthiourea and sodium diethyl dithiocarbamate, more effectively by the former (Table 1).

### DISCUSSION

Polyphenoloxidase activity in banana fruits has been extensively studied by Palmer (1963, 1964) as it causes enzymic oxidation of dopamine (3, 4-dihydroxyphenylethylamine) resulting characteristic browning of healthy banana. The present investigation shows that considerable increase in activity of this enzyme together with peroxidase result

following infection by the tested fungus. This is in accordance with similar observations by other workers in other host-pathogen interactions (Fehrmann and Dimond 1967; Jennings et al. 1969; Chattopadhyay and Nandi 1976). Increase in activities of the enzymes from early stages of infection indicates a triggering off in their syntheses which continues up to a certain period and then slows down to some extent. Complete absence of polyphenoloxidase and only some peroxidase in the mycelia in vitro does not exclude the possibility of their pathogenic contribution during host infection.

Increased activities of the enzymes were due to their increased syntheses. This finding was further substantiated from results of experiments with different inhibitors of protein synthesis. Level of peroxidase in healthy tissues remained fairly uniform throughout the experimental period apparently due to a low turnover. However, as expected, cycloheximide caused blocking or inhibition to a great extent of the peroxidase activity increase in response to infection.

Palmer (1964) observed an immediate inhibition of banana polyphenoloxidase activity by phenylthiourea ( $10^{-3}$  M) but a considerably delayed inhibition after addition of sodium diethyldithiocarbamate ( $10^{-3}$  M). In the present study, although the tested concentration ( $6\times10^{-3}$  M) of the two inhibitors was sufficiently high to cause strong inhibition of the enzyme, comparable results a stronger effect of phenylthiourea was also noted. Since banana polyphenoloxidase, like other phenolases, is a copper metaloenzyme, phenylthiourea is presumed to form a complex in some fashion with the copper on the active site of the enzyme and thus cause inhibition more effectively than other chelating agents (Palmer 1964).

In course of pathogenesis, activities of these oxidases have been found to be higher in the infected resistant tissues than in the infected susceptible one or the uninfected healthy ones (Tomiyama 1963). Since the degree of defence reaction in host is often known to be parallel to the amount of the enzyme, higher enzyme activity shows stronger host resistance. From this point of view, 'champa' variety seems to possess comparatively greater resistance against the test fungus than the other cultivar.

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Aktywność enzymów w owocach bananów gnijących pod wpływem Botryodiplodia theobromae Pat.

#### Streszczenie

Badano aktywność peroksydazy i oksydazy polifenolowej w owocach dwu odmian bananów — 'champa' i 'kanthali', które gniły pod wpływem Botryodiplodia theobromae Pat. Enzymy te wykazywały dużo silniejszą aktywność w zarażonych niż w zdrowych tkankach. Wzmożoną aktywność peroksydazy hamował cykloheksimid. Aktywność oksydazy fenolowej hamowała również obecność fenylotiomocznika i dwuetylodwutiokarbamanianu sodowego; wpływ tego ostatniego był jednakże słabszy. Wzmożona aktywność enzymów zdaje się być spowodowana ich bardziej intensywną syntezą. W kulturze in vitro grzyb wykazywał tylko aktywność peroksydazy.