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PEROXIDASE ACTIVITY IN RICE LEAVES INFECTED WITH *HELMINTHOSPORIUM ORYZAE* IN RELATION TO LESION MATURATION

By

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Two-week-old seedlings of two rice varieties, resistant CH13 and susceptible Benibhog, were inoculated with a conidial suspension of *Helminthosporium oryzae*. The resistant host exhibited greater peroxidase activity than the susceptible one when infected with the pathogen. Enzyme activities were measured 0, 24, 48, 72, 96 and 120 h after infection. Peroxidase activity in diseased leaves bearing young lesions, *i.e.*, 24 h after inoculation, was not appreciably different from that in non-infected leaves. At the intermediate and mature stages of lesion development, *i.e.*, 72 and 120 h after inoculation, a marked increase in peroxidase activity was observed. The pattern of increased enzyme activity was similar in both varieties but the whole event proceeded faster in the resistant variety. No marked change in peroxidase activity in healthy leaves of both the varieties was noticed with increase of seedling age.

KEY WORDS: Helminthosporium oryzae; Oryza sativa; brown spot disease of rice; peroxidase activity.

INTRODUCTION

Helminthosporium oryzae, the causative organism of brown spot disease of rice, is characterized by lesions which are sharply delimited within a short time. Resistance is determined by two sets of factors: *(a)* the number of lesions concerned with penetration and initial establishment of infection, and *(b)* lesion expansion, *i.e.*, size of lesions, which is controlled by internal factors. Lesions may reach their maximum size 96 and 120 h after inoculation. Several factors may be involved in inducing resistance to lesion expansion (2, 3, 12, 13, 14) on young leaves following infection. Changes in peroxidase activity may be responsible for determining resistance or susceptibility to a given pathogen (4, 5, 11, 15, 16). In other cases, increased peroxidase activity may be associated with degenerative processes caused by infection. The present approach is to examine the pattern of change in peroxidase activity in extracts of diseased leaves at different stages of lesion maturation in relation to the activity found in extracts from healthy leaves of comparable age.

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MATERIALS AND METHODS

Plant culture

Rice seeds of resistant (CH13) and susceptible (Benibhog) varieties were sown in earthern pots filled with sun-dried field soil. Tap water was added until the emergence of seedlings. The plants were grown under controlled conditions at 25° ± 1°C and watered twice daily. The seedlings were in the early 2-leaf stage with the second leaf fully expanded 15 days after emergence.

Inoculation

Conidial suspension of *H. oryzae* was obtained from mycelium cultured on a potato-dextrose agar medium for 10-15 days. The density of spores in distilled water was adjusted to 2.5 x 10^5 per milliliter. This suspension was sprayed on plants with a hand atomizer; control plants were sprayed with distilled water. The plants were incubated in a humid atmosphere for 24 hours and then transferred to growth chambers at 25° ± 1°C.

Infection value

To assay the infection value, size and total number of spots of a definite number of leaf samples, usually 25 leaves of almost similar size were taken. The spots were classified into three groups in relation to size of lesions, namely, small (flecks), up to 0.5-1 x 0.5 mm in size; medium (medium eye-shaped spots), up to 2 x 1 mm; and large (large eye-shaped spots), up to 2-3 x 1-1.5 mm.

For each small spot on a leaf an arbitrary value of 0.25 was ascribed and similarly, values of 0.5 and 1 were ascribed for medium and large spots, respectively. The calculated value was divided by the total number of leaves and the result obtained represents the number of lesions per leaf.

Plant enzyme preparation

Fresh leaf (0.5 g) was ground at 0°C in 3 ml of 0.05 M tris buffer pH 8.0, in a glass mortar. A small quantity of quartz sand was added to facilitate grinding and the triturated sample was centrifuged at 20,000 rpm for 15 min at 0°C. The supernatant was passed through a Sephadex column (G-100) and then dialysed in 0.01 M tris buffer pH 8.0 for 24 h. The dialysate was used as the enzyme source.

Peroxidase assay

To 5 ml of 0.5 M pyrogallol reagent in a 1-cm-diameter colorimeter tube, 1.0 ml of purified enzyme extract was added. The tube was inverted for thorough mixing of the contents and inserted in a Bausch and Lomb Spectronic-20 colorimeter at 420 mμ. The colorimeter galvanometer was then adjusted to zero optical density (O.D.) and 0.5 ml of 1% H_2O_2 was added rapidly to the tube. The tube was inverted once, and immediately reinserted into the colorimeter. The change in O.D. between 0.2 and 0.4 was measured at 420 mμ and timed by a stop watch. A change in absorption by 0.01/min at 420 mμ was accepted as a unit of enzyme activity.

Reagents

1. H_2O_2, 1%.

2. Pyrogallol reagent – made up immediately before use from *(a)* pyrogallol stock solution, 5 M and *(b)* phosphate buffer, pH 6.0, 0.6 M. Ten ml of (*a*) and 12.5 ml of *(b)* were mixed and made up to 100 ml with distilled water. All solutions were refrigerated.

RESULTS

Infection value

Maximum spread of lesions took place in the susceptible variety, Benibhog. The top-most leaves were resistant compared with their respective second leaves from the top. Lesions reached maximum to near maximum size 96 h after inoculation. In the resistant variety, CH13, number as well as size of lesions were less in comparison with the susceptible one, as evidenced by low infection values (Table 1). Symptoms on both hosts occurred initially as small brownish areas at the site of infection, first becoming visible 24 h after inoculation. There were marked visible differences between the lesions of the two hosts 48 h after incubation.

TABLE 1

INFECTION VALUES* OF BROWN SPOT DISEASE ON TWO VARIETIES OF RICE AT DIFFERENT TIMES AFTER INOCULATION

Variety	*Leaf position*	*Hours after inoculation*			
		48	72	96	120
Benibhog	Top-most leaf	3.5	5.6	6.6	6.8
	Second leaf from top	4.7	9.5	15.3	15.9
CH13	Top-most leaf	2.0	2.8	3.7	3.7
	Second leaf from top	3.7	5.7	7.6	7.7

* See Materials and Methods.

Enzyme activity

During the early phase of pathogenesis, which coincided with the incipient phase of lesion expansion, there was no significant rise in peroxidase activity. A marked increase in the enzyme activity was noted as from the intermediate stages of disease development, namely, maturation of lesions (Table 2).

TABLE 2

CHANGES IN PEROXIDASE ACTIVITY IN RICE LEAVES INFECTED WITH *HELMINTHOSPORIUM ORYZAE*

(Change in peroxidase activity was expressed by changes in the quantity of active enzyme units; a change in absorption (O.D.) by 0.01 per minute at 420 mμ was considered as a unit of enzyme activity.)

Leaf position	*Variety*	*Treatment*	*Number of active enzyme units present/g of fresh tissue* Hours after inoculation					
			0	24	48	72	96	120
Top-most leaf	Benibhog	Control	27.3	22.5	30.0	25.0	15.0	20.0
		Infected		35.0	75.0	150.0	169.0	200.0
	CH13	Control	36.0	40.0	45.0	32.0	35.0	50.0
		Infected		55.0	100.0	142.8	207.0	300.0
Second leaf from top	Benibhog	Control	32.5	30.0	25.0	30.0	30.0	35.0
		Infected		40.0	100.0	171.5	200.0	230.8
	CH13	Control	42.5	40.0	45.0	40.0	75.0	42.5
		Infected		65.0	132.5	240.0	267.5	312.5

Top-most leaf: Critical Difference (C.D.) at 5% level = 7.44; C.D. at 1% level = 9.89.
Second leaf from top: C.D. at 5% level = 7.12; C.D. at 1% level = 9.45.

The leaves of the resistant variety showed at all comparable stages much higher peroxidase activity than those of the susceptible one. Similarly, peroxidase activity was more pronounced in the second than in top-most leaves. The pattern of rise was similar for both the varieties but the whole event proceeded more rapidly in the resistant host-pathogen combination (the slope of the curve was much steeper). In healthy non-infected controls, no such increase in enzyme activity could be noticed.

Studies of lesion expansion showed that increase in peroxidase activity was correlated with disease progress. The first measured difference in peroxidase activity at 24 h after inoculation was higher in the infected tissue than in the non-infected control. With increasing time after inoculation, peroxidase activity became higher in the infected tissue of both varieties than in their respective healthy controls. At 120 h after infection, peroxidase activity in infected resistant leaves was approximately 7 to 8 times that of the respective non-infected control.

DISCUSSION

The results presented here are similar to other cases in which increased peroxidase activity has been reported following infection (6, 7, 8, 9, 10). Increased enzyme activity may result from *de novo* synthesis of the enzyme or its release from bound form (1).

During the early phases of pathogenesis there was no significant increase in peroxidase activity. A marked increase in the enzyme activity was observed at the intermediate stage of lesion maturation. It is therefore suggested that increase in enzyme activity was associated with the progress of the disease.

As peroxidase plays an important role in the defence mechanism against the disease, the tissue surrounding necrotic spots is influenced, resulting in *de novo* synthesis or activation of the enzyme. It may be concluded, however, that peroxidase alone can not account for resistance but that it produces a physiological status in the host rendering it inhospitable for the fungus, and thereby contributes to resistance.

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