PROTEINASE ACTIVITY IN RELATION TO LEAF SPOT OF RICE

Leaf spot disease of rice incited by *Helminthosporium oryzae* Breda de Haan is characterized by necrotic lesions on leaf surface. The lesions that appear in leaf spots are mainly composed of injured host tissues likely to be resulted from pathogen-produced proteolytic enzymes[1]. In a previous publication[2] from this laboratory, the implication of proteolysis in rice leaves infected with *H. oryzae* has been emphasized. The present investigation relates to studies made to find out the importance of pathogen-produced proteinase in host-parasite interaction.

A virulent strain of *H. oryzae* was grown on liquid medium[3], containing 1·5% casein. Cell free, crude enzyme was obtained by filtration of the culture medium followed by centrifugation (3,000 × g) at 4° C for 10 minutes. Rice plants of N.C. 678 variety (susceptible to *Helminthosporium* leaf spot) were artificially infected after four weeks of growth in pot cultures[4] with the same fungal strain. Necrotic regions of infected leaves and the corresponding tissues of healthy leaves were homogenized separately in 0·9% sodium chloride solution under ice-cold condition. Each preparation was centrifuged (3,000 × g) at 4° C for 10 minutes and the supernatant fluid was used as the enzyme source. Proteinase activity was assayed according to the method of Marks and Lajtha[5,6]. The assay system contained 100–150 μg of enzyme, 30 μ moles of phosphate buffer (pH 7·0) and 1 mg of bovine serum albumin (Final vol. 1 ml). Ninhydrin-positive substances released as a result of enzymic activity was measured by following the procedure of Samyn *et al.*[7]. Protein content was estimated by the method of Lowry *et al.*[8].

The results in Table I show that proteinase is highly active in the culture filtrates of *H. oryzae*, maximum activity being observed in 12 days old cultures. It is further noted that the extracts of both healthy and infected leaves exhibit proteinase activity which is always higher in the infected leaves, most significant difference being noticed on the 12th day following inoculation.

The quantitative differences in proteinase activity in the healthy and infected leaves implicate the involvement of the enzyme in host-parasite interaction. The enhanced enzymic activity in the infected leaves may possibly be due to pathogen-produced proteinase as apparent from strong proteolytic activity in the culture filtrates of *H. oryzae*.

TABLE I

Proteinase activity in culture filtrates of Helminthosporium oryzae *and in healthy and infected rice leaves of N.C. 678 variety*

Source	Specific activity*				
	Days following inoculation				
	4	8	12	16	20
Culture filtrates ..	1·98±0·16	3·23±0·28	5·40±0·51	3·86±0·36	2·71±0·15
Healthy leaves ..	0·56±0·06	0·63±0·06	0·74±0·07	0·79±0·08	0·82±0·07
Infected leaves ..	0·88±0·09	1·91±0·20	4·21±0·45	2·62±0·28	1·45±0·16

* Specific activity has been expressed as μ moles amino group released/mg. protein/hour. Each value represents an average of five separate determinations ± S.E.M.

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1. Husain, A. and Kelman, A., In *Plant Pathology*, Vol. I, Ed., J. G. Horsfall and A. E., Dimond, Academic Press, New York, 1959, p. 143.
2. Mukherjee, P. K. and Ghosh, J. J., *Phytochemistry*, 1972, 11, 3119.
3. Swinbume, T. R. and Corden, M. E., *J. gen. Microbiol.*, 1969, 55, 75.
4. Ganguly, D. and Padmanabhan, S. Y., *Indian Phytopath.*, 1959, 12, 99.
5. Marks, N. and Lajtha, A., *Biochem., J.*, 1963, 89, 438.
6. — and —, *Ibid.*, 1965, 97, 74.
7. Samyn, W., Carton, D. and Hooft, C., *Clin. Chim. Acta*, 1970, 28, 83.
8. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, 193, 265.