

ROLE OF PROTEINS AND CALCIUM IN RESISTANCE OF RICE TO INFECTION BY *HELMINTHOSPORIUM ORYZAE*

P. K. MUKHERJEE and J. J. GHOSH

Department of Biochemistry, University of Calcutta, Calcutta-19, India

(Revised Received 13 May 1972. Accepted 23 June 1972)

Key Word Index—*Oryza sativa*; Gramineae; cell walls; protein; calcium; resistance to *Helminthosporium oryzae*.

Abstract—The pectic substances, proteins and calcium in the cell wall preparations of healthy rice leaves of Co. 20 (resistant), T. 141 (resistant), N.C. 1281 (susceptible) and N.C. 678 (susceptible) varieties were measured. Although there is no significant variation in the content of pectic substances, the concentrations of proteins and calcium are higher in the resistant varieties than those in the susceptible varieties, implicating conformational differences in the cell wall structure of resistant varieties.

INTRODUCTION

AT THE primary stage of infection, the main target of a pathogen is to overcome the cell wall barrier of the host. That the middle lamella of rice leaf cell wall is affected in infection by *Helminthosporium oryzae* is evident from a microscopic study.¹ Field studies have revealed that, while certain varieties of rice plants are highly susceptible to infection by *H. oryzae*, there are other varieties which are more or less resistant to such infection. Hence it can be presumed that there may exist some differences in cell wall composition between the resistant and the susceptible varieties of rice plants. Pectic substances, proteins and calcium are known to be the important components of cell walls, especially of middle lamellae. The association of calcium with pectic substances in relation to resistance against fungal infection has been suggested by a number of workers.²⁻⁴ In the present investigation, the concentrations of pectic substances, proteins and calcium in the rice leaf cell walls of four known varieties have been estimated.

RESULTS AND DISCUSSION

Considering the importance of cell wall constituents in the resistance to infection, concentrations of pectic substances, proteins and calcium in the cell walls of rice leaves have been estimated (Table 1).

Results of analyses of different cell wall constituents reveal that although the variation in the content of pectic substances is insignificant among the four varieties studied, the concentrations of proteins and calcium are higher in the resistant varieties than those found

¹ K. SATO and M. SAKAMOTO, *Rep. Inst. Agric. Tohoku Univ.* **11**, 97 (1960).

² L. V. EDGINGTON, M. E. CORDEN and A. E. DIMOND, *Phytopathol.* **51**, 179 (1961).

³ B. J. DEVERALL and R. K. S. WOOD, *Ann. appl. Biol.* **49**, 461 (1961).

⁴ D. F. BATEMAN and S. V. BEER, *Phytopathol.* **55**, 204 (1965).

in the susceptible varieties (Table 1). Ito and Fujiwara^{5,6} have shown that calcium is the most abundant metal in the cell walls of rice leaves. Although part of the wall-bound calcium exists as pectate, pectate alone should not be considered to be related to structural integrity of the cell walls. Calcium associated with other cell wall constituents is also important. Ginzburg⁷ has obtained evidence for a protein-gel structure cross-linked by calcium and other polyvalent cations in the intercellular matrix of plant tissue. Pectolytic and proteolytic enzymes, which generally appear during infection processes, do not readily attack complexes formed by pectic polymers or pectic polymers and proteins linked by polyvalent cations.⁸ The implication of pectolytic and proteolytic enzymes in the rice leaves infected with *H. oryzae* has also been realized.⁹ It is, therefore, likely that in the resistant varieties, pectic substances have conformational differences which enable more reactive groups to form cross linkages with increased levels of Ca^{2+} and proteins, conferring increased resistance to attack by *H. oryzae*.

TABLE 1. CONCENTRATION OF PECTIC SUBSTANCES, PROTEINS AND CALCIUM IN LEAF CELL WALLS OF FOUR VARIETIES OF RICE*

Rice varieties Code No.	Type	Pectic substances	Proteins	Calcium
Co. 20	Resistant	12.9 \pm 0.6	43.8 \pm 2.0	5.52 \pm 0.25
T. 141	Resistant	12.7 \pm 0.5	39.1 \pm 1.8	5.60 \pm 0.25
N.C. 1281	Susceptible	11.8 \pm 0.5	30.6 \pm 1.4	3.62 \pm 0.17
N.C. 678	Susceptible	12.1 \pm 0.5	27.8 \pm 1.2	3.24 \pm 0.15

* Results have been expressed in mg/g dry wall materials. Each value represents an average of five separate determinations \pm s.e. of mean.

EXPERIMENTAL

Materials. Rice seeds of Co. 20 and T. 141 varieties were collected from the Central Rice Research Institute Cuttack, India, and those of N.C. 1281 and N.C. 678 varieties were obtained from the State Rice Research Station, Chinsura, West Bengal, India.

Methods. The rice seedlings were raised in 6 in. pots under similar conditions described by Ganguly and Padmanabhan.¹⁰ Healthy leaves were collected after 4 weeks of growth. Preparation of cell walls was made by the method of Ito and Fujiwara.⁵ Concentration of pectic substances was determined colorimetrically by the methods of McComb and McCready,¹¹ and McCready and McComb;¹² HCl at pH 2.0 was used for extraction. Protein content was calculated from the total nitrogen value estimated by the method of Ma and Zuazaga.¹³ Concentration of calcium was determined according to the titrimetric method of Ghosh and Roy.¹⁴

Acknowledgement—Our thanks are due to Dr. S. Y. Padmanabhan, Dr. N. K. Chakravarti and Dr. D. P. Bhattacharya of Central Rice Research Institute, Cuttack for their kind interest in this work.

⁵ A. ITO and A. FUJIWARA, *Plant & Cell Physiol.* **8**, 409 (1967).

⁶ A. ITO and A. FUJIWARA, *Plant & Cell Physiol.* **9**, 433 (1968).

⁷ B. Z. GINZBURG, *J. Exptl Bot.* **12**, 85 (1961).

⁸ J. WALLACE, J. KUC and H. N. DRAUDT, *Phytopathol.* **52**, 1023 (1962).

⁹ P. K. MUKHERJEE, Ph.D. Thesis, University of Calcutta (1970).

¹⁰ D. GANGULY and S. Y. PADMANABHAN, *Indian Phytopathol.* **12**, 99 (1959).

¹¹ A. MCCOMB and R. M. MCCREADY, *Analyt. Chem.* **24**, 1630 (1952).

¹² R. M. MCCREADY and A. MCCOMB, *Analyt. Chem.* **24**, 1986 (1952).

¹³ T. S. MA and G. ZUAZAGA, *Ind. Engng Chem. Analyst. Edn.* **14**, 180 (1942).

¹⁴ A. K. GHOSH and K. L. ROY, *Naturwissenschaften* **24**, 644 (1955).