

## FLUORESCENT ANTIBODY STAINING FOR DETECTION OF COCONUT ROOT WILT ANTIGEN

FLUORESCENT antibody (FA) technique serves as a useful tool for detecting *viral antigens* and their localization *in situ*. Coons *et al.*<sup>1</sup> developed this technique. Since then the technique has been widely employed for both plants and animals, infected with fungi, bacteria, viruses and mycoplasma<sup>2-5</sup>. Among plants considerable work has been done with atleast five viruses, namely, tobacco mosaic<sup>6</sup>, wound tumor<sup>7</sup>, southern bean mosaic<sup>8</sup>, cauliflower mosaic<sup>9</sup> and narcissus yellow stripe<sup>10</sup>.

The present report describes the use of FA technique in the detection of coconut root wilt antigen in the epidermal cells of tobacco. The coconut root wilt virus<sup>11</sup> was transmitted and maintained on *Nicotiana tabacum* cv. White Burley. The antigen was purified after Summanwar *et al.*<sup>11</sup>. The concentrated antigen was administered to rabbits weekly by four intravenous injections and two intramuscular injections (with Freund's adjuvant). The blood sample collected by bleeding the rabbits after 15 days following the last injection, was allowed to clot at room temperature for four hours and stored overnight in a refrigerator for clot to shrink. The serum was clarified by low speed centrifugation at 4,000 rpm for 15 minutes. The antiserum reacted specifically with the coconut root wilt antigen and had a titre of 1:1024.

Following Spendlove<sup>12</sup>, the  $\gamma$ -globulins were precipitated with equal volume of 3.2-N ammonium sulphate. The precipitated globulins were recovered by centrifugation at 1,000 g for 30 minutes. The precipitate was dissolved in distilled water equal to the original volume of the serum and reprecipitated with ammonium sulphate as before. Residual sulphate was removed by overnight dialysis using distilled water. The pH of the globulin solution was raised to 9.0 using 0.5 M carbonate-bicarbonate buffer. 50 mg of fluorescein isothiocyanate (FITC) was added per gram protein in cold (4° C) and kept for 18 hours. The unconjugated dye was removed by overnight dialysis against buffer.



FIG. 1. Diseased epidermal peel of *N. tabacum* cv. White Burley. Arrows indicate labelling of the coconut root wilt antigen with FITC.

Epidermal peels were taken from healthy and diseased tobacco leaves infiltrated in acetone,

The peel, were mounted on glass slides and allowed to almost dry. Labelled FITC was added to the glass slides and allowed to remain for 4-6 hours. The peels were then washed with buffered saline pH 7.5 (0.01 M PO<sub>4</sub> and 0.15 M NaCl) and mounted in the same buffer.

Observations were made using super-high-pressure mercury bulb HBO 200 with BG 3 and BG 12 light filters. Pictures were taken using suppression OG 1 filters. Healthy peels showed a uniformly dull orange fluorescence. On the other hand, the diseased peels showed an overall orange fluorescence, interspersed with deep apple green spots in the cytoplasm of the cells (Fig. 1, arrow marked—white spots). The deep apple green spots marked the labelling of the coconut root wilt antigen in the systemically infected tobacco epidermal cells.

The authors are thankful to Dr. S. P. Raychaudhuri, Head, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for facilities and encouragement.

Division of Mycology and Plant Pathology, I.A.R.I., New Delhi 110012, August 25, 1975.

R. PADMA,  
ARVIND S. SUMMANWAR,  
K. JAGADISH CHANDRA\*

\* Present address: Department of Plant Pathology, Indian Institute for Horticultural Research, Bangalore.

1. Coons, A. H., Creech, H. J., Jones, R. N. and Berliner, E., *J. Immunol.*, 1942, 45 159.
2. Kumar, D. and Patton, R. F., *Phytopathology*, 1964, 54, 898.
3. Anger, J. G. and Shalla, T. A., *Ibid.*, 1975, 65, 493.
4. Sinha, R. C., *Current Trends in Plant Pathology*, 1974, p. 99.
5. Kumar, D., Ghosh, S. K., Raychaudhuri, S. P., Nariani, T. K. and Varma, A., *Curr. Sci.*, 1975, 44, 170.
6. Schramm, G. and Rotger, B., *Z. Naturforsch.*, 1959, 146, 510.
7. Nagraj, A. N., *Virology*, 1965, 25, 133.
8. Worley, J. F. and Schneider, I. R., *Phytopathology*, 1963, 53, 1255.
9. Day, M. F. and Venablis, D. G., *Australian J. Biol. Sci.*, 1961, 14, 187.
10. Cremer, M. C. and Vander J. A. Velean, *Neth. J. Pl. Path.*, 1964, 72, 105.
11. Summanwar, A. S., Raychaudhuri, S. P., Jagadish Chandra, K., Nam Prakash and Lal, S. B., *Curr. Sci.*, 1969, 38, 208.
12. Spendlove, R. S., In *Methods in Virology*, (K. Maramorsch and H. Kuprowski, Eds.) Vol. III, Academic Press, New York, 1967, p. 457.