Enzyme dot blot assay: A diagnostic tool for detecting rice tungro virus infection

RICE tungro virus (RTV) disease is caused by infection of two different viruses, rice tungro spherical virus (RTSV) and rice tungro bacilli form virus (RTBV) (ref. 1). Both RTSV and RTBV are transmitted in a semi-persistent manner by rice green leaf hopper *Nephotettix virescens*. RTSV infection causes mild or indistinct symptoms. The spherical viral particles are isometric, have a diameter of 30 nm and contain a single-stranded DNA genome of more than 10 kb. RTBV infection, however, causes severity of symptomatology as evident from yellowing of leaves and stunted growth and the symptoms are accentuated in plants co-infected with RTSV[2-4]. The bacilli form particles have a model length of 130 nm and width of 30 nm and can only be acquired by plants from leafhoppers in association with RTSV. The RTBV genome is 8 kb (ref. 5) and is interrupted by two discontinuities which map at specific sites on each strand. Neither RTBV nor its DNA is transmitted mechanically into rice plants. The virus contains two major proteins with molecular weight of 37 and 33 kD.

The method of early detection being mainly serological depends on highly purified viral particles to develop the antiserum. Serological methods, though specific and sensitive enough to detect viruses even in small quantity of samples, the virus purification and obtaining good quality antiserum is rather difficult and resource intensive. Therefore, a study was undertaken to develop simpler, cost-effective technique to detect RTV infection at early stages, using enzyme-based dot-blot assay.

Seven to ten-day-old seedlings of seven genotypes, viz., Taichung (Native) 1, ARC 11554 (IRRI Acc.# 21473), ARC 12596 (# 22176), DWA 8, PTB 8 (# 6201), Palasithari 601 (# 12069), and Utri Merah (# 16682) were inoculated with RTV through the viruliferous vector. Leaf samples were collected after 20 days when seedlings expressed clear symptoms, especially in T(N)1. The samples were extracted in Tris-HCl (0.5 M, pH 6.8), centrifuged (9000 *g*) for 20 min at 4°C and supernatant was used for enzyme dot-blot assay.

Dot-blot assay was performed by blotting the extract onto Whatman No. 1 filter paper by dot-blotting manifold, and staining for alcohol dehydrogenase (ADH), acid phosphatase (ACP), glutamate dehydrogenase (GDH), esterase (EST) and malate dehydrogenase (MDH) (Figure 1). Of the five enzymes studied, ADH and ACP showed difference in activity between the healthy and the infected samples. In case of ACP, the colour changed from brown to blue. Furthermore, depending upon the severity of infection, there was variation in the intensity of colour. Other enzymes such as EST, MDH and GDH did not give consistent results. To check these, proteins were separated on 7.5% non-denaturing acrylamide gel under anionic condition using constant current (20 mA) and gels were stained for ADH and ACP. In both the cases, all the healthy samples showed

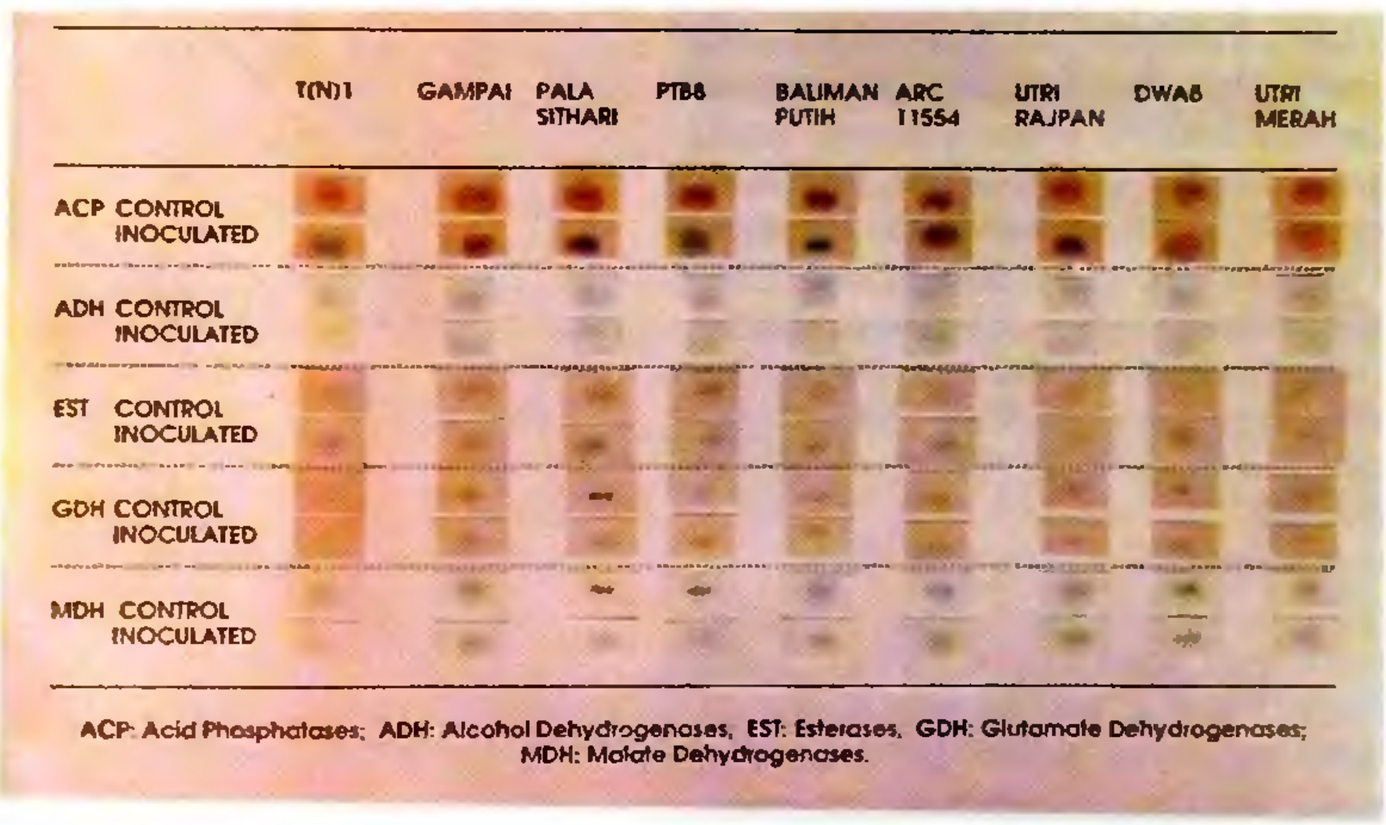

Figure 1. Change in enzyme activities on infection with RTV (20 days after infection).

Table 1. ELISA readings (average of 3 replications) of RTBV and RTSV infected plant samples from seven rice genotypes

Sl. no.	Genotype[a]	RTSV	RTBV	Score[b] Phenotypic	Score[b] Dot-blot
1	Infected (I)	0.64	0.50	+++	+++
	Healthy (H)	0.08	0.06		
2	I	0.56	0.49	+	+
	H	0.07	0.04		
3	I	0.14	0.05	–	+
	H	0.01	0.00		
4	I	0.11	0.35	+	+
	H	0.09	0.03		
5	I	0.24	0.05	++	++
	H	0.01	0.05		
6	I	0.33	0.49	+	+
	H	0.21	0.04		
7	I	0.01	0.34	+	+
	H	0.0	0.06		

[a] 1: Taichung (Native) 1; 2: ARC 11554; 3: ARC 12596; 4: DWA 8; 5: PTB 8; 6: Palasithari 601; 7: Utri Merah.
[b] +: Degree of symptoms expressed; –: No symptoms expressed.

one band and no bands were observed in the infected samples. With other enzymes, the difference was more of nature of change in the intensities of the staining rather than one in the pattern. The difference in colour in the dot-blots was, however, not detectable when sampling was done 7 days after inoculation.

These results were further verified by comparing them with those of ELISA. Lyophilized antisera against RTBV and RTSV obtained from Koganezawa (IRRI) were purified by precipitation with ammonium sulphate and by passing through DEAE cellulose column. Purified antibodies were used for making conjugate with alkaline phosphatase enzyme. Double antibody sandwich ELISA was performed for RTBV (B) and RTSV (S). Average reading of the 'S' and 'B' forms in infected and healthy plants and their phenotypic scores (Table 1) shows that 'B' form showed stronger reaction than 'S' form. Furthermore, infected samples showing higher reading also showed higher degree of change in colour on dot-blots, compared to the healthy ones.

Although this technique being of qualitative nature cannot be compared with techniques such as ELISA, it can be employed as a reliable, low-cost technique for rapid screening of large number of samples.

1. Saito, Y., Iwaki, M. S. and Usugi, T., *Ann. Phytopathol. Soc. Jpn.*, 1976, **43**, 375.
2. Hibino, H., *Ann. Phytopathol. Soc. Jpn.*, 1983, **49**, 545–553.
3. Hibino, H., *Plant Dis.*, 1983, **67**, 774–777.
4. Cabanatan, P. Q. and Hibino, H., *Philipp. J. Phytopathol.*, 1985, **21**, 103–109.
5. Jones, M. C., Gough, K., Dasgupta, I. *et al.*, *Gen. Virol.*, 1991, **72**, 757–761.

P. ARTI
V. V. SHENOY*
A. GHOSH**
N. P. SARMA
E. A. SIDDIQ†

Directorate of Rice Research, Rajendranagar, Hyderabad 500 030, India
**University of Agricultural Sciences, Dharwad 580 005, India*
***Indian Institute of Pulses Research, Kanpur 208 024, India*
†ICAR, Krishi Bhavan, New Delhi 110 001, India

Possible occurrence of a Fabry–Perot type of interference filter in a fish eye-lens

The eyes of fish are constructed on general vertebrate plan with cornea, lens and retina. But unlike terrestrial vertebrates, the lens in fish acts as the principal refractive structure, since little refraction occurs at the corneal surface, whose refractive index is approximately equal to that of water.

The function of lens in both 'camera type' and 'compound type' eye appears to be extensive and varied. It may act as a broad band anti-reflection device, may help in reflection of specific wavelength from regions behind the receptive cells, can play a role in selective light absorption and also may act as an interference filter in the eye[1–5]. The optical phenomena exhibited by lens have generally been correlated with the specialization in morphological and anatomical features, and also in organic macromolecular complexes[1–5]. Surprisingly, the possible involvement of inorganic components in these has so far not been explored in detail, although many inorganic elements are known to possess important optical properties with varied applications[6]. The conventional techniques, such as atomic absorption spectroscopy used in the past for elemental analysis of lens and other ocular tissues could not provide information on distribution patterns of elements and their localization in different components of a particular structure.

Electron microscopy–energy dispersive X-ray spectroscopy (EM–EDS), which uses electron optics and characteristic X-rays emitted from elements, appears to be the best device which meets the above criteria. Recent studies from this laboratory could explain some unexplored optical phenomena in relation to the localized distribution of certain elements in fish lens[7,8].

Here we report with the help of scanning electron microscopy and energy dispersive X-ray micro-analysis, a unique structural arrangement of lens components and the localized distribution of the metal, aluminium in the lens, in a way that resembles a Fabry–Perot type of interference filter. The transmission curve of the lens also shows similarity with the said interference filter.

Lens taken out from the eye of a fresh water fish, *Hilsa ilsa* was placed in a carbon-coated brass stub and was cut into two halves with a sharp razor blade. Since the lens is structurally stable, no preparatory technique was needed and it was studied by direct mounting. No mould or embedding medium was used for cutting the hard lens. The sectioned lens was secured to the stub with adhesive tape in such a way that the cut surface would face the electron beam of the microscope. Care was taken to avoid contact of the cut surface with any source of elemental contamination. The samples were air-dried, coated with carbon in a JEE-4X vacuum evaporator (Jeol), and studied in the secondary electron emission and X-ray mode of the scanning electron microscope (JSM-35 CF, Jeol). Energy dispersive X-ray micro-analysis was carried out with EDAX system (AN 10000, Linc Analytical) equipped with a Si (Li) high resolution detector. The operating parameters selected were as follows: accelerating voltage = 20 kV, tilt = 45°, elevation = 0.00°, azimuthal angle = 0.00°. The counting time for each analysis was 100 s, the count rate was 2500 cps and the dead time was 25%. The transmission curve of the lens was obtained using the UV-visible double beam spectrophotometer (U-2000, Hitachi). The sections of lens were placed on a glass cover-slip and the transmittance spectra were