Detection of Rice Transitory Yellowing Virus (RTYV) in Infected Rice Plants and Insect Vectors by Simplified ELISA

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Rice transitory yellowing virus (RTYV) transmitted by *Nephotettix* leafhoppers in a persistent manner causes yellowing of the lower leaves and reduced tillering in infected rice plants¹⁾. Symptoms of the disease closely resemble those of tungro, orange leaf disease and physiological disorders²⁾. This disease has been reported to occur in Taiwan¹⁾, Okinawa³⁾, China and Thailand⁴⁾. Methods to detect the virus include a transmission test using leafhoppers, electron microscopic (EM) examination, and western blot analysis using peroxidase conjugated concanavalin A (Con A)⁵⁾. However, each method has its shortcoming; the transmission test takes over one month to obtain results, EM is not suitable for mass inspection, and Con A can not detect the virus antigen in insect vectors. Recently, serological detection of virus antigens has become possible owing to successful virus purification which enables specific antiserum production⁶⁾. This paper describes the establishment of a mass inspection method for the detection of RTYV in rice plants and viruliferous insects by comparing results from the passive hemagglutination test (PHA), latex flocculation method (LF), enzyme-linked immunosorbent assay (ELISA) and simplified ELISA⁶⁾.

RTYV-infected rice plants were maintained and proliferated using viruliferous *Nephotettix nigropictus* which had been fed on infected rice plants (cv. Koshihikari). The antiserum against the purified virus (T. Hayashi, unpublished) had a titer of 1 : 500 in the precipitin ring interface test.

We first examined PHA. Formalinized sheep erythrocytes⁷⁾ were sensitized with antiserum^{8,9)}. The virus antigens in viruliferous insects were successfully detected (data not shown). However, we could not detect them in infected plants due to the inhibition of hemagglutination in PHA.

For latex sensitization with antiserum, we used the procedure of Bercks and Querfurth¹⁰⁾.

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LF could not detect virus antigens in infected leaves due to non-specific flocculation, but it could detect the virus antigens in viruliferous insects when 0.01 M citrate buffer containing 0.85% NaCl, pH 6.0 (CBS), was used as an extraction buffer.

Thus, these two methods could not detect the virus antigens in infected plants, but could those in viruliferous insects. Tests using the ELISA described below showed that these insects exhibited A_{410} value >1.5 , *i.e.*, the sensitivity was low in both methods. We therefore do not recommend these methods for the detection of RTYV.

Standard ELISA¹¹⁾ was tested using microplates coated with 5 μ g/ml of γ -globulin, and the conjugate of alkaline phosphatase with γ -globulin diluted to 1 : 400. The absorbance at 410 nm was measured by MR580 microplate autoreader (Dynatech Laboratories, Inc.) 1 hr after the addition of substrate.

The effect of the homogenizing buffers on the detection of the virus antigens was studied. Infected rice leaves or viruliferous insects were macerated in 0.01 M phosphate buffer, pH 7.0 (PB), 0.01 M PB containing 0.85% NaCl and 0.05% Tween 20, pH 7.4 (PBS-T), CBS, pH 6.0 or 0.01 M Tris-HCl buffer, pH 8.0, diluted with respective buffers and assayed by ELISA. Although there was little difference in the sensitivity among buffers for the detection of virus antigens, PBS-T showed the lowest non-specific reaction to healthy homogenates for both plants and insects. Based on these results, PBS-T was used as the extraction and dilution buffer.

Using the ELISA method described above, the virus was detected in the roots, tillering nodes, stalks and leaves of infected rice plants at dilutions of up to 6,400, 25,600, 25,600 and 25,600 times, respectively. This method was also used to detect viruliferous insects. Individual leafhoppers were homogenized with 0.4 ml PBS-T. While individual insects exposed to virus for 20 days gave A_{410} values ranging continuously from 0 to >1.5 , all unexposed leafhoppers gave A_{410} values <0.08 (data not shown, refer Table 1). The insects which gave an A_{410} value ≥ 0.1 were counted as viruliferous insects. No significant difference in A_{410} values was observed between insects of different sex. The maximum dilution giving a positive reaction for individual viruliferous insect was 4,260 times.

Simplified ELISA in which the number of manipulations is reduced by simultaneous incubation of virus antigen and conjugate with solid-phase antibody was tested¹²⁾. The γ -globulin concentration and conjugate used were the same as those for standard ELISA. The effect of the incubation time on the sensitivity of simplified ELISA was studied. A 50 μ l conjugate diluted to 1 : 100 was mixed with a 150 μ l sample in each well making the final conjugate dilution 1 : 400. When the sample-conjugate mixtures were incubated for 0.5, 1 and 2 hr at 37 C, the dilution end points of the infected leaves were 12,800, 12,800 and 25,600 times, respectively. These results demonstrate that the sensitivity of the simplified ELISA is the same as that of the standard ELISA. The simplified version was also used to detect RTYV in viruliferous insects. The methods were the same as those reported before⁶⁾ except that the incubation period was 30 min. While the A_{410} values for insects not exposed to the virus were less than 0.05, those for virus-exposed insects ranged continuously (Table 1).

To confirm the reliability of the simplified ELISA for detecting virus antigens in insect vectors, the infectivity of viruliferous insects was examined and each insect was then tested by simplified ELISA. For the transmission test and detection of virus antigens in insect vectors, viruliferous insects were individually transferred onto test seedlings for 2 days, then used for serological examination as described above. The insects that transmitted virus in the assays were counted as active transmitters. Rice cultivar Taichung Native 1 (TN1) and Koshihikari were used as test plants. As shown in Table 2, all insects that transmitted the virus gave positive reactions and all insects that gave negative reactions were non-transmitters. These results prove the reliability of the simplified ELISA for the detection of viruliferous insects of RTYV.

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Table 1. Detection of RTYV in individual leafhoppers by simplified ELISA

A_{410} values in ELISA	Number of leafhoppers ^{a)}			
	Unexposed		Exposed	
	male	female	male	female
0.00-0.05	37	29	110	110
0.05-0.1	0	0	13	13
0.1-0.2	0	0	4	13
0.2-0.4	0	0	4	4
0.4-0.6	0	0	13	11
0.6-0.8	0	0	16	6
0.8-1.0	0	0	6	10
1.0-1.5	0	0	24	13
1.5-	0	0	2	8

a) *Nephotettix nigropictus*.

Table 2. The transmissibility of RTYV by leafhoppers which showed positive or negative reaction in the simplified ELISA

Cv. of rice	Exp. no.	No. of insects				
		Simplified ELISA ^{a)}			Transmission test	
		Total	Positive	Negative	Transmitters	Non-transmitters
TN1	1	50	27	23	10 0	17 23
	2 ^{b)}	50	20	30	5 0	15 30
Koshihikari	1	50	17	33	10 0	7 33
	2	50	19	31	13 0	6 31

a) The leafhoppers which gave A_{410} value of ≥ 0.1 in the simplified ELISA were counted as positive.

b) The acquisition feeding period was 5 days.

Based on our results, we recommend the simplified ELISA for the detection of RTYV because the process is much simpler than the standard ELISA. Also, plates coated with γ -globulin could be successfully stored at 4 C, and the results could be obtained within 2 hr. Simplified ELISA should be very useful for epidemiological studies in field inspection of RTYV.

和 文 摘 要

高橋義行・大村敏博・林 隆治・匠原監一郎・土崎常男：簡易ELISA法によるイネ黄葉ウイルス(RTYV)の罹病イネおよび保毒虫からの検出

血清学的手法を用いて RTYV の検出を試みた。赤血球凝集反応法およびラテックス凝集反応法の場合、ウイルスは罹病イネからはそれぞれ反応抑制や非特異反応のために検出できなかったが、保毒クロスジマグロヨコバイからは虫体内のウイルス濃度が高い場合にのみ検出できた。ELISA 法では罹病イネ葉の25,600倍希釈まで、保毒虫からは4,260倍希釈まで検出できた。簡易ELISA法は、短時間の処理でELISA法と同等の感度を示した。伝搬試験の結果と比較したところ、本ウイルスを伝搬した虫は必ず簡易ELISA法で検出され、さらに伝搬試験では検出できなかった保毒虫からもウイルス検出が可能であった。簡易ELISA法は、短時間内に多数の保毒虫検定を終了することができるのでRTYVの疫学的調査に有効な手法であると考えられる。

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