DETECTION OF RICE VIRUSES IN PLANTS AND INDIVIDUAL INSECT VECTORS BY SEROLOGICAL METHODS

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ABSTRACT

Latex flocculation test (LF) and enzyme-linked immunosorbent assay (ELISA) were employed to detect virus antigens in infected rice plants and viruliferous insects. Reciprocal of dilution end points for infected plants tested 40 days after inoculation was 160, 40, 5,120, 5,120, 10,240, 40 and 80 for rice gall dwarf (RGDV) rice ragged stunt (RRSV), rice dwarf RDV), rice grassy stunt (RSV), rice stripe (RSV), rice tungro bacilliform (RTBV) and rice tungro spherical (RTSV) viruses, respectively by LF, and was 5,120, 640 and 1,280 for RGDV, RTBV and RTSV, respectively by ELISA. Virus antigens were detected by LF from the following individual insects: *Nephotettix nigropictus* viruliferous for RGRV or RDV, *Nilaparvata lugens* viruliferous for RGSV and *Laodelphax striatellus* viruliferous for RSV.

Introduction

Virus-infected rice plants have been identified mainly by visual symptoms, or by insect transmission of the virus to assay plants. Visual symptoms are not always specific in the field because more than one virus may cause similar symptoms in rice plants and many non pathogenic disorders such as nutritional deficiencies, excess water after drought, or insect injury may cause virus-like symptoms. Insect transmission assays are laborious as well as time-consuming; some persistent viruses require 2 wk incubation in the vector and another approximately 2-3 wk for symptom development in test plants. Moreover, virus-free vector populations must be available at various stages for transmission assays. On the other hand, serological assays are usually specific and, in some cases, sensitive enough to detect virus even in individual insects. However, serological techniques are often complex and may require special facilities.

For rapid identification of rice viruses and for epidemiological studies of rice virus diseases, serological tests with the following attributes are needed: 1) A simple method which can be used in the laboratory without sophisticated equipment; 2) Able to detect virus antigens in crude sap; 3) Sensitive enough to detect virus antigens in individual insects; 4) Applicable for many samples in a short time. In this paper, the application of the latex flocculation test (LF) was introduced to detect rice gall dwarf (RGDV), rice dwarf (RDV), rice ragged stunt (RRSV), rice grassy stunt (RGSV), rice stripe (RSV), rice tungro bacilliform (RTBV) and rice tungro spherical (RTSV) viruses. Enzyme-linked immunosorbent assay (ELISA) was also applied to detect virus antigens with a low titer detected by LF. Methods employed in this study can be referred to Bercks and Querfurth (1971), Clark and Adams (1977) and Omura *et al.*, (1984).

Detection of virus antigens in rice plants by LF

As shown in Table 1, all the virus antigens were detected by LF. Maximum titers of the antigens were obtained 30-50 days after inoculation and little change in titer occurred during this period in all the viruses tested. Antigen concentrations were higher in root than in leaves of plants infected with RGDV or RRSV. Antigen titer was identical regardless of the presence of galls on the leaf used for RGDV or RRSV. Antigen concentrations were higher in leaves than in roots in the

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Table 1 Detection of virus antigens in rice plants at various times after inoculation by latex flocculation test[a)]

Virus	Infected material	Days after inoculation 2	30	40	50	60	70
RGDV	Leaf	20[b)]	20	20	40	40	40
	Root	80	160	160	160	160	160
RRSV	Leaf	10	10	10	10	0	0
	Root	20	40	40	20	20	10
RDV	Leaf	1,280	5,120	5,120	5,120	5,120	2,560
RGSV	Leaf	5,120	5,120	5,120	5,120	5,120	5,120
RSV	Leaf	10,240	10,240	10,240	10,240	10,240	10,240
RTBV	Leaf	20	80	40	40	40	40
RTSV	Leaf	40	80	80	80	80	80

a) No flocculation was observed in any of the healthy controls for all the viruses tested.
b) Reciprocal of the highest dilution with positive reaction.
(Data from Omura *et al.*, 1984).

rest of the viruses (data not shown). Antigen concentration decreased with increasing time after inoculation in RRSV-infected plants. These plants recovered from the disease with the lapse of time. Antigen titers of plants infected with RDV, RGSV or RSV were much higher as compared to those infected with RGDV, RRSV, RTBV or RTSV.

Detection of virus antigen in insect vectors by LF

Results were identical in all the experiments both using crude insect macerates or supernatant after centrifugation. As shown in Table 2, antigen titers did not vary significantly 15 days after the acquisition access for all the viruses with a positive reaction. The results were negative when 0.05 M Tris-HCl, pH 7.2 which contained 0.02% (W/V) polyvinylpyrrolidone (Tris-PVP) was used as extraction buffer for RGDV and RDV. Use of 0.01 M phosphate buffer, pH 7.0 containing 0.01 M $MgCl_2$ (PB-Mg) improved flocculation, however, non specific flocculation occurred in healthy controls. The addition of Tween-20 to PB-Mg dispersed non specific aggregates without affecting the specific reaction, as previously described with potato (Fribourg, 1978). Flocculation was clearly observed for RGSV and RSV when individual insects were homogenized either with Tris-PVP or PB-Mg-Tween. Reactions were clear when individual insects were homogenized in 0.1 ml buffer for RGSV and RSV. However, no reaction occurred for RGDV and RDV when individual insects were homogenized in the same amount of buffer. Flocculation occurred when more than 0.4 ml buffer was used to homogenize single insect for RGDV and RDV. For RRSV no flocculation occurred under a variety of test conditions.

The reason for the inability to detect RRSV antigen in viruliferous insect was ascribed to the interference of the flocculation by insect homogenate (data not shown).

Table 2 Detection of virus antigen in viruliferous insects at various times after acquisition access by latex flocculation test[a)]

Virus	Insect	Sex	Days after acquisition access started					
			5	10	15	25	30	35
RGDV	*Nephotettix nigropictus*	Female			32	32		32
		Nymph	0[b)]	8[c)]				
		Male			16	16		16
RRSV	*Nilaparvata lugens*	Female			0	0		0
		Nymph	0	0				
		Male			0	0		0
RDV	*N. nigropictus*	Female			32	64		32
		Nymph	0	8				
		Male			16	32		16
RGSV	*N. lugens*	Female			64	64	64	
		Nymph	0	16				
		Male			32	16	16	
RSV	*Laodelphax striatellus*	Female			16	16	16	
		Nymph	0	4				
		Male			8	8	8	

a) No flocculation was observed in any of the healthy controls for all the viruses tested.
b) Negative reaction.
c) Reciprocal of the highest dilutions of crushed insects.
(Data from Omura *et al.*, 1984).

Detection of virus antigens by ELISA

As shown in Table 3, RGDV, RTBV and RTSV were detected up to a 5,120, 640 and 1,280 dilution, respectively from the infected plants. The results were identical using both crude macerates or supernatant after centrifugation.

Table 3 Detection of RGDV, RTBV and RTSV in infected rice plants by enzyme-linked immunosorbent assay

Virus	Dilution										
	20	40	80	160	320	640	1,280	2,560	5,120	10,240	C[a)]
RGDV	+[b)]	+	+	+	+	+	+	+	+	–[c)]	–
RTBV	+	+	+	+	+	+	–	–	–	–	–
RTSV	+	+	+	+	+	+	+	–	–	–	–

a) Control: buffer.
b) Positive reaction.
c) Negative reaction.

Conclusion

LF was found to be an excellent and convenient serodiagnostic method to detect antigens of rice viruses in both plant and insect vectors except for RRSV in insect vector. ELISA proved to be a very sensitive method to detect virus antigens with low titer detected by LF. So far, ELISA is recommended to detect RRSV in insect vector (Hibino and Kimura, 1982).

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Discussion

Reddy, D.V.R. (ICRISAT): I must mention that we encountered problems with the latex flocculation test. Regarding the cost of ELISA, if you consider the high dilution at which you can use the antiserum and the large number of samples that can be handled at one time, ELISA is certainly not more expensive than any other conventional serological tests. As you may know, ELISA has been recently modified and can be performed in three hours.

Answer: With regard to the cost of ELISA this depends on the quality of the antiserum. When the dilution of the conjugate is above 3,000 times, the cost of ELISA is similar to that of the latex flocculation test. However when the antiserum is of poor quality and the dilution of the conjugate amounts to 400 times, the cost of ELISA is twice as high as that of the latex flocculation test. In the simplified ELISA it is sometimes difficult to distinguish between positive and negative reactions. The use of monoclonal antibodies which do not react with healthy controls may alleviate this shortcoming. The simplified ELISA appears to be more complex than the latex flocculation test.

Makkouk, K.M. (ICARDA): The cost of ELISA can be reduced by the repeated use of the plates (5-6 times) by washing.

Anjaneyulu, A. (India): How long can you preserve the sensitivity of sensitized latex stored at room temperature, in the refrigerator and in the deep freezer?

Answer: Latex sensitized with antiserum to rice stripe virus can be preserved for three years when stored in the refrigerator with sodium azide. In the case of RTSV and RTBV, data indicate that the sensitivity can be maintained for a period of one and a half years.