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Citation	Journal of the Faculty of Agriculture, Hokkaido University, 63(3), 277-284
Issue Date	1987-12
Doc URL	<a href="http://hdl.handle.net/2115/13064">http://hdl.handle.net/2115/13064</a>
Type	bulletin (article)
File Information	63(3)_p277-284.pdf



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## COMPARISON OF DIRECT AND INDIRECT ELISA AMONG DIFFERENT ISOLATES OF BYMV

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Received July 13, 1987

### Introduction

The disease which causes severe stem necrosis and sometimes leads to the death of French bean cv. Ōfuku has been recorded frequently in Hokkaido. UYEDA *et al.*<sup>12)</sup> reported that it was caused by necrotic strain of bean yellow mosaic virus (BYMV).

Up to now, four isolates of BYMV, No. 30, No. 36, No. 55 and CS, showing different symptoms on french beans were isolated in Hokkaido. The isolates No. 30 and No. 36 show necrotic spots on the inoculated leaves, mosaic and severe necrosis on the upper leaves of broad bean. When the isolate No. 55 was inoculated to broad bean, similar symptoms to those inoculated by the isolates No. 30 and No. 36, slowly developed, accompanying clear vein clearing on the upper leaves. The isolate CS shows typical mosaic symptom on the upper leaves but did not show necrosis on the inoculated leaves and stems.

During field survey for BYMV using both indexing plants and enzyme-linked immunosorbent assay (ELISA)<sup>9)</sup>, we noticed isolates of BYMV inducing mosaic symptoms on broad bean and French bean could not be detected by ELISA using antiserum to isolate No. 36. This lead us to investigate strain specificity of ELISA.

This paper describes the serological characteristics of four isolates of BYMV by three different ELISA.

### Materials and Methods

The following isolates of BYMV maintained in our laboratory were used; No. 30 (UYEDA *et al.*<sup>10)</sup>), No. 36 (SUGANO,<sup>9)</sup> No. 55 (NAKASONE,<sup>8)</sup> and

TABLE 1. Host range and symptoms of each isolates

plant species	isolates			
	No. 30	No. 36	No. 55	CS
<i>Phaseolus vulgaris</i> L.				
cv. Top crop	N	N	N, M	CS
cv. Ōfuku	N	N	N	M
<i>Vicia faba</i> L.				
cv. Wase-soramame	N, M	N, M	N, M	M
<i>Pisum sativum</i> L.				
cv. Ōmidori	N, M	N	Y	M
<i>Glycine max</i> Merrill	M	M	—	—
<i>Phaseolus angularis</i> (Willd.) Wight	N, M	+	—	—
<i>Vigna sesquipedalis</i> Fruwirth	CS	—	—	—
<i>Trifolium repens</i> L.	M	M	M	N. T.
<i>T. pratense</i> L.	N	—	N. T.	N. T.
<i>Nicotiana tabacum</i> L.	+	+	—	—
<i>Chenopodium amaranticolor</i> Coste & Ryne	L, S	L, S	L, S	N. T.

N: necrosis M: mosaic CS: chlorotic spot Y: yellowing L, S: primary lesion and systemic infection +: latent infection —: no symptoms

N. t.: not tested

Data in literature cited: KUME<sup>5)</sup>, NAKASONE<sup>6)</sup>, SUGANO<sup>9)</sup>, UYEDA *et al.*<sup>10)</sup>

CS (KUME,<sup>5)</sup>). Host range and their symptoms of these four isolates were shown in Table 1.

They were maintained and propagated in broad bean (*Vicia faba* L. cv. "wase-soramame"). The virus was purified according to UYEDA *et al.*<sup>10)</sup>.

Rabbits were immunized by two intramuscular injections of 100 µg of the purified virus emulsified in Freund's complete adjuvant, followed by intravenous injections of 40 µg. The titers of each antiserum were shown in Table 2.

Double antibody sandwich direct ELISA (DAS-ELISA) was performed by a method described by CLARK and ADAMS<sup>1)</sup>, and antigen absorption direct ELISA (AAD-ELISA) and antigen absorption indirect ELISA (AAI-ELISA) were done by the methods of CROOK and PAYNE<sup>2)</sup>. Concentration of antibody used was 5 µg/ml and alkaline phosphatase labelled rabbit IgG enzyme conjugate and alkaline phosphatase labelled sheep anti-rabbit IgG enzyme conjugate (Capel lot #20577) were used at 400 folds dilution with PBS-T.

The absorbance at 405 nm was read with a micro-immunoplate photometer (Corona Co. Ltd.).

## Results

### 1. Precipitin ring test

Results of the precipitin ring test showed that all isolates reacted homologous and heterologous antisera. However, the isolates could be differentiated by their reciprocals of dilution end points.

The dilution end points of isolates No. 30 and 36 were indistinguishable at homologous and heterologous reactions, differing only 1/2 dilution at most (Table 2). The dilution end point obtained in CS isolate are apparently distinct from those of both isolates No. 30 and No. 36 in heterologous reaction. Isolate No. 55 reacted somewhat less than isolates No. 30 and 36, but considerably higher than isolate CS to No. 30 and 36 antisera.

TABLE 2. Reciprocals of dilution end-points of the four isolates in precipitin ring test

antisera to	isolates			
	No. 30	No. 36	No. 55	CS
No. 30	512	256	256	64
No. 36	512	512	256	64
No. 55	256	128	512	128
CS	128	64	64	512

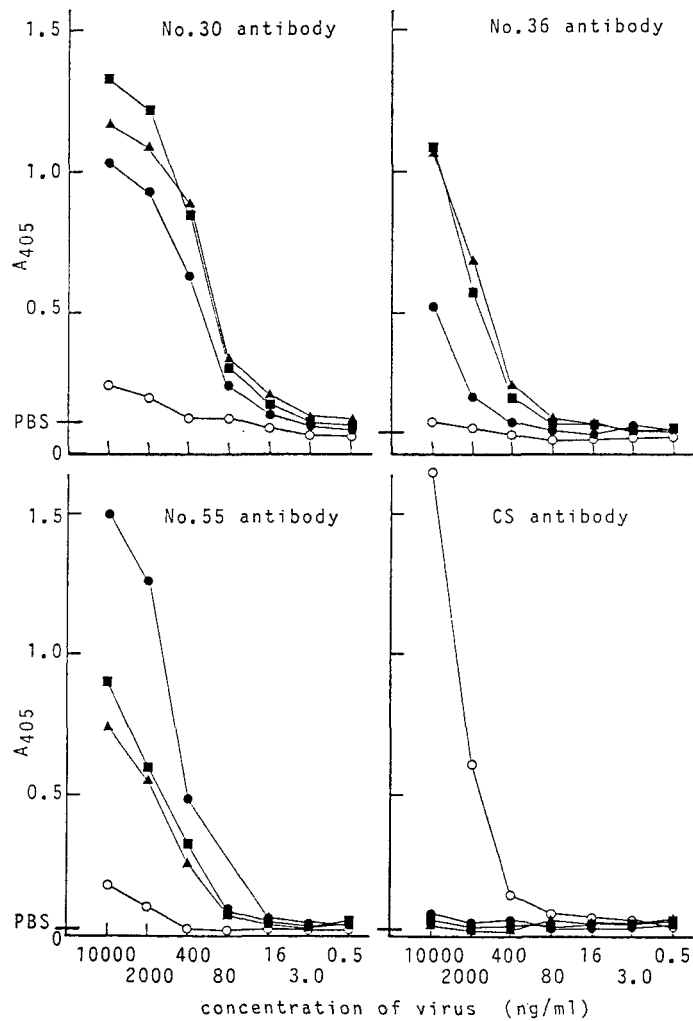
### 2. ELISA

Results of DAS-ELISA as shown in Fig. 1, showed remarkable difference in heterologous reaction from those obtained in precipitin ring test as shown in Table 2. Isolates No. 30 and No. 36 did not react with CS antiserum and vice versa. Isolate No. 55 also did not react with CS antiserum and was differentiated from isolates No. 30 and No. 36 with slightly lower absorbance values in heterologous reactions.

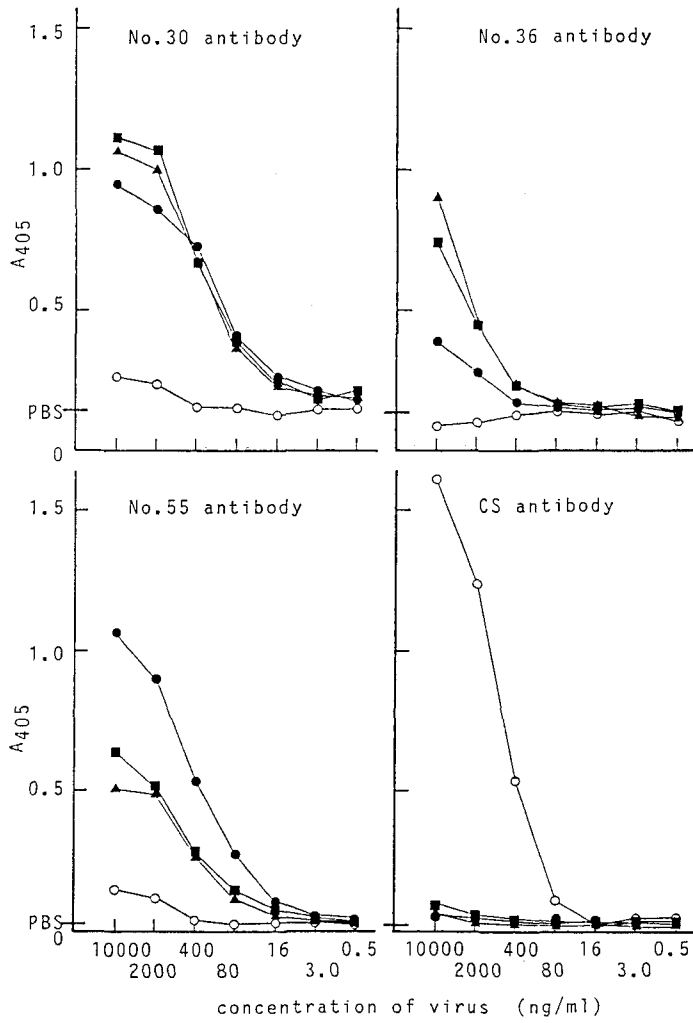
Similar results obtained by AAD-ELISA, as shown in Fig. 2, indicated that the reactivities of these four viruses to each of four antisera did not significantly differ from DAS-ELISA.

In contrast to DAS-ELISA and AAD-ELISA, reactions of the isolates to the antisera by AAI-ELISA as shown in Fig. 3, were less specific. Isolates No. 30 and 36 reacted weakly with CS antiserum, whereas they did not at all by DAS- or AAD-ELISA. Isolate CS also reacted with No. 30 and

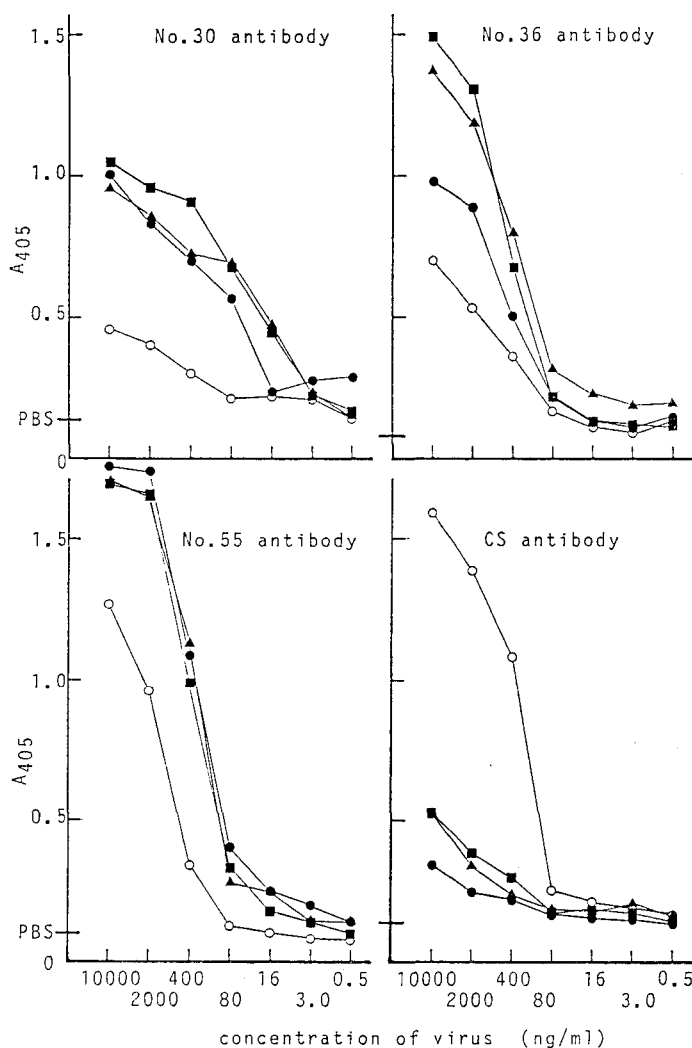
36 antisera. Absorbancy of isolate CS by AAI-ELISA against No. 55 anti-serum was considerably higher than that of DAS-ELISA.



**Fig. 1.** Double antibody sandwich ELISA (DAS-ELISA)  
Antigens: No. 30 (■), No. 36 (▲), No. 55 (●), CS (○)



**Fig. 2.** Antigen absorption direct ELISA (AAD-ELISA)  
Antigens: No. 30 (■), No. 36 (▲), No. 55 (●), CS (○)



**Fig. 3.** Antigen absorption indirect ELISA (AAI-ELISA)

Antigens: No. 30 (■), No. 36 (▲), No. 55 (●), CS (○)

### Discussion

DAS-ELISA shows very high specificity and differentiates closely related viruses which were not detected by conventional serological methods (KOENIG<sup>39</sup>). ROCHOW and CHARMICHAEL<sup>8</sup> reported that the strain specificities were very strong by DAS-ELISA using four strains of barley yellow dwarf virus (BYDV). Our results also showed the similar strain differentiation between

the necrotic isolates and CS strain, in contrast to the results of the precipitin ring test. These findings suggested that DAS-ELISA can be reliably used to differentiate serological viral strains.

AAD-ELISA was reported to have more broad specificity than DAS-ELISA (CROOK and PAYNE<sup>2</sup>), but in our experiment the specificities were not so different in both ELISA.

It has been reported that AAI-ELISA had "wide spectrum" specificity (CROOK and PAYNE<sup>2</sup>, van REGENMORTEL and BUCHARD<sup>13</sup>, KOENIG<sup>4</sup>). This decrease in specificity was considered to be induced by the difference in the binding strength between conjugated antibodies and "native" antibodies (KOENIG<sup>3</sup>, van REGENMORTEL and BUCHARD<sup>13</sup>). As conjugated antibodies have lower binding strength than "native" antibodies, the former are easily dissociated with antigens. So, in DAS-ELISA, antibodies which react heterologous antigen probably are mostly dissociated. In contrast, AAI-ELISA provides "native" antibodies, and lower degree of "heterologous dissociation" detecting heterologous antigen.

Our results of AAI-ELISA also showed the wider spectrum in specificity. The weak reaction values between the necrotic isolates and CS strain in the two direct methods became stronger to the detectable level. These results suggested that this method is of great importance for detecting closely related viruses in the fields. Combination of DAS- and AAI-ELISA methods can facilitate the diagnosis of virus and virus strains in samples collected from the fields.

The host ranges and symptoms of the isolates and the strain were shown in Table 1. UYEDA *et al.*<sup>10</sup> suggested that isolate No. 30, No. 36 and No. 55 belonged to BYMV necrotic strain for their characteristic necrotic symptoms and their serological relationships. But the symptoms on broad bean of isolate No. 55 are slightly different from those of isolates No. 30 and No. 36. Isolate No. 55 appears mosaic first and follows necrotic symptom, but in the later two, necrosis quickly appears associated with mosaic symptoms.

The results of our precipitin ring test and ELISA showed that isolates No. 30 and No. 36 reacted identically and that isolate No. 55 reacted differently from No. 30 and 36. These results indicated that isolate No. 55 was different from isolates No. 30 and 36.

### Summary

Three variations of enzyme-linked immunosorbent assay (ELISA) such as direct double antibody sandwich (DAS), antigen absorption direct (AAD)



and antigen absorption indirect (AAI) methods, were tested by using antibodies to bean yellow mosaic virus (BYMV) isolates No. 30, No. 36, No. 55 and CS. DAS- and AAI-ELISA showed similar reaction specificity and can be used for differentiation of the isolates. AAD-ELISA showed stronger heterologous reaction. The relationships between isolates No. 30, No. 36 and No. 55 were discussed.

### Acknowledgements

This work was supported in part by the grant from the Japan Legume Crops Fund Association (Nihon-Mame-ru Kikin Kyokai) in 1983-1985.

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### **Explanation of plates**

Plate 1. Necrosis and necrotic lesion on broad bean affected by BYMV No. 30.

2. Necrotic lesion on broad bean affected by BYMV No. 36.

3. Mosaic and necrosis on broad bean affected by BYMV No. 55.

4. Mosaic on broad bean affected by BYMV CS.

