

日本に発生するインゲンマメ黄斑モザイクウイルス(BYMV)の病原性と血清学的性状の比較

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Biological and Serological Comparisons of Bean Yellow Mosaic Virus (BYMV) Isolates in Japan

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Abstract

To compare biological and serological reactions of 28 bean yellow mosaic virus (BYMV) isolates collected from different hosts and locations in Japan, 15 different bean (*Phaseolus vulgaris*) cultivars were inoculated with the 28 isolates. Four biological variations (pathotypes I-IV) were observed among the 28 BYMV isolates. Pathotype I systemically infected only one bean cultivar, Honkintoki, out of the 15 cultivars studied. Pathotype II infected systemically Honkintoki, Kentucky Wonder and four other cultivars. Pathotype III infected systemically Honkintoki, Kentucky Wonder, Master Piece and four other cultivars. Pathotype IV infected systemically all 15 bean cultivars. Pathotype II also showed higher seed-transmissibility in broad bean (*Vicia faba*) than other pathotypes. The 28 BYMV isolates also varied serologically in their reactions in TAS-ELISA to 16 monoclonal antibodies (MAbs) produced against a BYMV isolate and two clover yellow vein virus (CIYVV) isolates. These reactions corresponded, to some degree, to biological variability. MAb-1F3 reacted with pathotypes I, II and III, and with one CIYVV isolate. MAb-2C4 reacted only with pathotype II. MAb-5F2 reacted with all BYMV and CIYVV isolates. MAb-2B4, -2C5, -3F9, -3F11, -4G8 and -4H9 reacted with all BYMV isolates in pathotypes II and III, and with some BYMV isolates in pathotypes I and IV. MAb-1A2 and -2H8 reacted strongly with pathotype III and with two CIYVV isolates. On the other hand, the differences in reactivity of six polyclonal antisera against four BYMV isolates and two CIYVV isolates in DAS-ELISA were not observed clearly. Our results indicate that the BYMV population in Japan is biologically and serologically variable and can be grouped into four pathotypes.

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Key words : bean yellow mosaic virus, clover yellow vein virus, host reaction, pathotype, monoclonal antibody, serology.

INTRODUCTION

Bean yellow mosaic virus (BYMV), first reported by Pierce²⁰, is distributed worldwide and causes severe disease in many leguminous and ornamental plants^{2-4,8,12,13,16,31,33}. The virus, now known to form a distinct subgroup (BYMV-subgroup) from other potyviruses, also includes clover yellow vein virus (CIYVV)^{2,21,34}. The identification of BYMV has been confusing because the BYMV-subgroup viruses are more similar to each other than to other potyviruses. Not only do they have very similar host ranges, they also induce the same type of cytoplasmic cylindrical inclusions and crystalline nuclear inclusions. They are very closely related serologically and their coat protein (CP) amino acid sequence identities are slightly higher (70-77%) than those observed between other potyviruses (38-70%)^{1-4,12,14,15,18,21,34,36}.

Although they have similar properties, numerous studies have shown biological and serological variabil-

ities among BYMV and CIYVV isolates from different parts of the world^{1-6,8,11,12,14,16,25}. Previously, we reported that CIYVV in Japan could be grouped into two strains on the basis of biological, serological and molecular properties. One of these strains was identified as CIYVV on the basis of coat protein sequence comparisons, in spite of its similarity to BYMV in host reactions²⁵.

Although BYMV has been an important pathogen of leguminous and ornamental plants in Japan for many years, only limited studies have assessed variation among BYMV isolates. Inouye¹² pointed out biological differences of BYMV isolates, including CIYVV isolates and classified BYMV isolates into strains B, P, O and N (N is now considered to be CIYVV). Kaneshige *et al.*¹⁶ further showed that BYMV-Cro4, isolated from *Crocus* sp. was biologically different from the four BYMV strains. Although biological variability of BYMV isolates in Japan has been reported, serological variability of BYMV isolates has not yet been examined. Information on the variability of BYMV is not only required for reliable diagnosis, but also bears on breeding for resis-

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tance to BYMV, as well as on the introduction of resistant crop genotypes for BYMV control.

In this study, we compared the biological and serological variability of BYMV isolates collected from different hosts and locations in Japan, identified four pathotypes of BYMV and described reactions of monoclonal antibodies to each isolate.

MATERIALS AND METHODS

Virus isolates and host reaction The 28 BYMV isolates and two CIYVV isolates used in this study. These isolates and their original hosts are shown in Table 1. All isolates of BYMV and CIYVV were passaged three times through *Chenopodium amaranticolor*, then propagated in broad bean (*Vicia faba*) plants. The upper leaves with mosaic of broad bean were stored at -80°C and used for inoculation tests. Each virus isolate was used for mechanical sap inoculation of *N. benthamiana*, *N. clevelandii*, *C. amaranticolor*, broad bean, 15 bean (*Phaseolus vulgaris*) cultivars and two pea (*Pisum sativum*) cultivars. The plants were grown in the

greenhouse at $22-25^{\circ}\text{C}$. All asymptomatic were tested 3-4 weeks after inoculation by back-inoculation to *C. amaranticolor*.

Seed-transmission test of broad bean Three broad bean cultivars, Ooshima-zairai, Sanuki-nagazaya and Ryousai-issun, were inoculated with 24 BYMV isolates in the greenhouse. After the appearance of mosaic symptoms, the broad bean plants were transplanted in a field, then sprayed with an insecticide at 10- to 15-day intervals until harvest. Harvested seeds were sown and grown in the greenhouse until the third or fourth node stage to observe the appearance of symptoms. Diseased plants were checked for BYMV infection by DAS-ELISA using polyclonal antiserum to BYMV-90-2.

Purification of viruses BYMV and CIYVV were propagated in broad bean and purified by the procedure outlined by Uyeda *et al.*³⁵⁾ except that CsSO_4 density gradient centrifugation was used instead of sucrose gradient centrifugation. SDS-denatured viruses (SDS-proteins) of BYMV-90-2, CIYVV-90-1 or CIYVV-92-3 were prepared by the procedure of Jordan *et al.*¹⁵⁾, with some modification. The purified virus was dissociated by

Table 1. Designation, host and place of origin of bean yellow mosaic virus (BYMV) and clover yellow vein virus (CIYVV) isolates used in the comparison studies

Isolate	Pathotype	Original host ^{a)}	Collection site in Japan	Source ^{b)}
BYMV- 92-2	I	broad bean	Kouchi	Author
G1		gladiolus	Unknown	I. Fujisawa
IbG		gladiolus	Ibaraki	Y. Takatsu ³⁷⁾
G2		gladiolus	Kagawa	Author
Gla3		gladiolus	Nagano	Author
A-2		Russell prairie gentian	Kouchi	S. Takeuchi ³¹⁾
Cro4		crocus	Nagoya	N. Inouye ¹⁶⁾
90-2	II	broad bean	Kagawa	Author ²³⁾
B		broad bean	Kagoshima	T. Inouye ¹²⁾
S		broad bean	Hokkaido	T. Hagita ⁹⁾
90-3		broad bean	Ehime	Author ²³⁾
90-5		broad bean	Tokushima	Author
94-1		broad bean	Kagawa	Author
94-2		broad bean	Ehime	Author
MB4		broad bean	Miyagi	S. Nakamura ¹⁹⁾
MB5		broad bean	Miyagi	S. Nakamura ¹⁹⁾
P	III	pea	Wakayama	T. Inouye ¹²⁾
102		red clover	Hokkaido	T. Hagita ⁹⁾
CS		red clover	Hokkaido	I. Uyeda ³⁴⁾
O	IV	bean	Hokkaido	T. Inouye ¹²⁾
92-1		red clover	Tokushima	Author
G		gladiolus	Nagano	N. Inouye ¹⁶⁾
Gla		gladiolus	Hokkaido	T. Hagita ⁹⁾
Gla4		gladiolus	Ibaraki	Author
Fri		freesia	Kagawa	Author
121		bean	Hokkaido	T. Hagita ⁹⁾
S-2		winged bean	Hukuoka	T. Ito ¹³⁾
B-1		Russell prairie gentian	Kouchi	S. Takeuchi ³¹⁾
CIYVV- 90-1		broad bean	Kagawa	Author ²⁴⁾
92-3		broad bean	Tokushima	Author ²⁵⁾

a) Original host means the plant in which the virus was initially found.

b) Source refers to the individual who supplied the isolate for these studies.

boiling for 3 min in 0.1% SDS. Intact virus particles were then removed by centrifugation at $160,000 \times g$ for 1.5 hr. These purified viruses and SDS-proteins were stored at -80°C .

Production of polyclonal antisera White rabbits were immunized with 1.0–3.0 mg/ml of purified viruses (BYMV-92-2, -90-2, -P, -O; CIYVV-90-1, -92-3) emulsified with equal volume of Freund's complete adjuvant. A second injection in incomplete adjuvant was given two weeks later, followed in two weeks by a third injection. For each injection, 2.0 ml of virus emulsion was injected into the thigh muscles. Finally, the rabbits were given a 1.0 mg/ml booster. One week after this final injection, the rabbits were bled.

Production of monoclonal antibodies (MAbs) Three 5-week-old male BALB/c mice were immunized by intraperitoneal injection of 100 μg of purified virus of BYMV-90-2 emulsified in Freund's complete adjuvant and were boosted 27 days later by an intravenous injection of the same amount of the purified virus in saline. At day 30, the spleen was removed and 0.9×10^8 cells were fused with the myeloma partner PAI using PEG 4000. Antibody-producing primary hybridoma cells were cloned twice by limiting dilution, and the hybridoma culture supernatant of each clone was used¹⁰⁾. In addition to MAbs to BYMV-90-2, five MAbs to CIYVV-90-1

(MAb-1A2, -1C8, -2H8, -3G9 and -5F2) and three MAbs to CIYVV-92-3 (MAb-2D6, -3H8 and -6F8) were also obtained²⁵⁾.

ELISA DAS (double antibody sandwich)-, TAS (indirect triple antibody sandwich)- and ACP (indirect antigen-coated plate)-ELISA were all used in this study.

In DAS-ELISA⁷⁾, Immulon I flat-bottom microplates (Dynatec Laboratories Inc.) were coated with 200 μl of anti-BYMV or anti-CIYVV rabbit IgG (1.0 $\mu\text{g}/\text{ml}$ in carbonate buffer, pH 9.6) for 4 hr at 37°C . After washing with phosphate-buffered saline, pH 7.2, containing 0.05% Tween-20 (PBS-T), 200 μl of antigen was added to triplicate wells and incubated overnight at 4°C . Antibody conjugates, diluted 1:800 for BYMV-P, BYMV-O and CIYVV-92-3 and diluted 1:1,600 for BYMV-92-2, BYMV-90-2 and CIYVV-90-1 in PBS-T, were added to triplicate wells and incubated for 4 hr at 37°C .

In TAS-ELISA¹⁵⁾, the plates were first coated with 200 μl of anti-BYMV or anti-CIYVV rabbit IgG (1.0 $\mu\text{g}/\text{ml}$ in carbonate buffer) for 4 hr at 37°C . After washing with PBS-T, 200 μl of antigen in PBS-T was added to triplicate wells and incubated overnight at 4°C . For ACP-ELISA¹⁵⁾, the plates were first coated with 200 μl of antigen in carbonate buffer and incubated overnight at 4°C . After washing, the antigen step was followed by adding 200 μl of antibody. Hybridoma culture super-

Table 2. Biological variability of 28 bean yellow mosaic virus (BYMV) isolates in Japan on the basis of symptom of 15 selected bean cultivars

Cultivar	Reaction patterns								
	1-0	1-1	1-2	2-0	3-0	4-0	4-1	4-2	4-3
Honkintoki	S ^{a)}	S	S	S	S	S	S	S	S
Kentucky Wonder	L	L	L	S	S	S	S	S	S
Kurodane-kinugasa	L	L	L	S	S	S	S	S	S
Shiro-kinugasa	L	L	L	S	S	S	S	S	S
Oo-tebou	L	L	L	S	S	S	L	S	S
Hime-tebou	L	L	L	S	S	S	L	S	S
Master Piece	L	L	L	L	S	S	S	S	S
Top Crop	L	L	L	L	L	S	S	S	S
Shuttle	L	L	L	L	L	S	S	S	S
Hodaka	L	L	L	L	L	S	L	S	S
Apollo	L	L	L	—	—	S	S	L	S
Morocco	L	L	L	L	L	S	S	L	L
Edogawa	L	S	L	L	L	S	S	S	S
Yamashiro-kurosando	L	L	L	L	L	S	S	S	L
Toramaru-uzura	L	L	S	L	L	S	S	S	S
Test isolate	G1 IbG A-2 Gla3 Cro4	G2	92-2	90-2 B S 90-3 90-5 94-1 94-2 MB4 MB5	P 102 CS	O G Gla4 Fri 121 S-2	Gla	92-1	B-1
Pathotype	I			II	III	IV			

a) S, L and — showed systemic infection, local infection and no infection, respectively.

natants of MAbs were diluted 1:100 in PBS-T. In the case of Mab-2C5, however, the culture supernatant was diluted 1:50 in PBS-T. After incubation for 4 hr at 37°C, bound antibodies were detected by goat-anti-mouse IgG or IgM conjugated to alkaline phosphatase (Sigma Chemical Co.), diluted 1:5,000 in PBS-T. For the last antibody step, the plates were incubated for 1 hr at 25°C. Enzyme reactions were developed 1 hr after the addition of 200 µl of substrate [*p*-nitrophenyl phosphate, disodium (Sigma Chemical Co.) at 1.0 mg/ml in 10% diethanolamine buffer, pH 9.8]. In the case of Mab-2C5, the incubation period for the enzyme reaction was 3 hr. These ELISAs utilized purified virus preparations at a final concentration of 100 ng/ml or crude extracts of virus-infected broad bean plants ground 1/100 (w/v) in PBS-T.

RESULTS

Host reaction

BYMV isolates in Japan can be classified into four

pathotypes (I-IV) based on the 15 bean cultivars' reactions (Table 2). Pathotype I could systemically infect only the universal susceptible one, Honkintoki. Pathotype II could systemically infect six bean cultivars, Honkintoki, Kentucky Wonder, Oo-tebou, Hime-tebou, Kurodane-kinugasa and Shiro-kinugasa. Pathotype III could infect seven out of the 15 bean cultivars including Honkintoki, Kentucky Wonder and Master Piece. Pathotype IV could systemically infect all 15 cultivars. Cultivars Honkintoki, Kentucky Wonder, Master Piece and Top Crop were selected as the representative cultivars to distinguish these pathotypes. Eight bean cultivars, Oo-tebou, Hime-tebou, Hodaka, Apollo, Morocco, Edogawa, Yamashiro-kurosando and Toramaru-uzura, showed different reaction to five BYMV isolates (G2, 92-2, Gla, 92-1 and B-1) as shown in Table 2.

A summary of other reactions of the test plants is shown in Table 3. Pathotype II caused either local or no infections on *N. benthamiana* and *N. clevelandii*. Pathotype III caused systemic infections on *N. benthamiana* and local infections on *N. clevelandii*. BYMV-90-2, -102,

Table 3. Biological variability of 28 bean yellow mosaic virus (BYMV) on the basis of symptom of selected differential hosts

Isolate	Pathotype	<i>Nicotiana benthamiana</i>	<i>Nicotiana clevelandii</i>	<i>Chenopodium amaranticolor</i>	<i>Vicia faba</i>	<i>Pisum sativum</i>	
						Sanjunchi-kinusaya	Wisconsin-Perfection
BYMV- 92-2	I	o/(Mo) a)	o/o	NS/-	CS/Mo, N	CS/Mo, N	-/-
G1		o/Mo	o/(Mo)	NS/-	CS/Mo	NS/Mo	-/-
IbG		o/Mo	o/(Mo)	CS/-	CS/Mo	o/Mo	-/-
A-2		o/Mo	o/CS	NS/-	CS/Mo	o/(Mo)	-/-
G2		o/(Mo)	o/o	NS/-	CS/Mo	CS/Mo	-/-
Gla3		o/(Mo)	o/o	NS/-	NS/Mo	CS/Mo, N	-/-
Cro4		o/Mo	o/o	NS/-	CS/Mo	CS /Mo	-/-
90-2	II	o/-	o/-	CS/Mo	o/Mo	o/(Mo)	-/-
B		-/-	o/-	CS/-	o/(Mo)	o/(Mo)	-/-
S		o/-	-/-	CS/-	o/Mo	o/Mo	-/-
90-3		-/-	o/-	CS/-	o/Mo	o/Mo	-/-
90-5		o/-	o/-	CS/-	o/Mo	o/(Mo)	-/-
94-1		-/-	o/-	CS/-	o/(Mo)	o/(Mo)	-/-
94-2		-/-	o/-	CS/-	o/(Mo)	o/(Mo)	-/-
MB4		-/-	o/-	NS/-	CS/Mo	NS/Mo, N, LN	-/-
MB5		-/-	o/-	CS/-	CS/Mo	o/(Mo)	-/-
P	III	o/o	o/-	CS/-	o/Mo	o/YMo	-/-
102		o/o	o/-	CS/Mo	CS/Mo	o/Mo	-/-
CS		o/(Mo)	o/-	CS/CS	o/Mo	o/Mo	-/-
O	IV	o/(Mo)	o/o	NS/Mo, N	o/(Mo)	o/(Mo)	-/-
92-1		o/Mo	o/o	CS/-	NS/Mo, N	o/Mo	-/-
G		o/(Mo)	CS/o	NS/Mo, NS	CS/Mo	CS/(Mo)	-/-
Gla		o/Mo	o/o	NS/-	CS/Mo, N	o/YMo	-/-
Gla4		o/Mo	o/o	NS/-	CS/Mo	CS/YMo	-/-
Fri		o/Mo	o/o	NS/-	CS/Mo	o/(Mo)	-/-
121		o/Mo	o/o	NS/-	o/(Mo)	o/(Mo)	o/(Mo)
S-2		o/Mo	o/o	NS/-	NS/Mo	o/YMo	-/-
B-1		o/o	o/o	NS/-	CS/Mo, N	NS/Mo, N, LN	-/-

a) Symptom abbreviation: CS, chlorotic spots; LN, lethal necrosis; Mo, mottle or mosaic; (Mo), weak mottle or mosaic; YMo, yellow mosaic; N, necrosis; NS, necrotic spots; o, latent infection; -, no infection. Symptoms on inoculated leaves are to the left of the slash, and symptoms in systemically infected leaves are to the right of the slash.

Table 4. Seed-transmissibilities of 24 bean yellow mosaic virus (BYMV) isolates in broad bean

Isolate	Pathotype	Broad bean cultivar		
		Ooshima-zairai	Sanuki-nagazaya	Ryousai-issun
BYMV- 92-2	I	2/210 (1.0) ^{a)}	0/310 (0.0)	0/135 (0.0)
G1		0/201 (0.0)	3/321 (0.9)	0/142 (0.0)
IbG		N.T. ^{b)}	N.T.	N.T.
G2		0/178 (0.0)	0/189 (0.0)	0/211 (0.0)
Gla3		0/121 (0.0)	2/217 (0.9)	0/168 (0.0)
A-2		0/237 (0.0)	0/211 (0.0)	0/109 (0.0)
Cro4		N.T.	N.T.	N.T.
90-2	II	18/431 (4.2)	11/347 (3.2)	6/185 (3.2)
B		15/387 (3.9)	8/473 (1.7)	4/211 (1.9)
S		1/271 (0.4)	12/341 (3.5)	9/218 (4.1)
90-3		0/424 (0.0)	2/312 (0.6)	5/158 (3.2)
90-5		0/347 (0.0)	5/127 (3.9)	3/78 (3.8)
94-1		4/268 (1.5)	3/128 (2.3)	3/195 (1.5)
94-2		3/211 (1.4)	6/246 (2.4)	4/208 (1.9)
MB4		2/68 (2.9)	2/183 (1.1)	4/211 (1.9)
MB5		0/100 (0.0)	13/341 (3.8)	8/257 (3.1)
P	III	0/136 (0.0)	0/173 (0.0)	0/216 (0.0)
102		1/130 (0.8)	0/186 (0.0)	2/265 (0.8)
CS		1/283 (0.4)	4/252 (1.6)	2/189 (1.1)
O	IV	0/337 (0.0)	0/319 (0.0)	0/612 (0.0)
92-1		0/241 (0.0)	0/214 (0.0)	2/134 (1.5)
G		0/237 (0.0)	0/311 (0.0)	0/138 (0.0)
Gla		0/361 (0.0)	1/181 (0.6)	0/118 (0.0)
Gla4		0/212 (0.0)	3/433 (0.7)	3/203 (1.5)
Fri		N.T.	N.T.	N.T.
121		1/113 (0.9)	0/211 (0.0)	0/139 (0.0)
S-2		N.T.	N.T.	N.T.
B-1		2/315 (0.6)	0/173 (0.0)	0/162 (0.0)

a) Seed-transmissibility showed number of seedlings showing symptoms/total seedlings examined and its percentage in parenthesis.

b) Not tested.

-CS, -O and -G caused systemic infections on *C. amaranticolor*, which did not associate with the pathotypes. Although all BYMV isolates could infect the pea cultivar Sanjunichi-kinusaya, only BYMV-121 could infect the pea cultivar Wisconsin-Perfection.

Seed-transmissibility in broad bean

Seed-transmissibility of 24 BYMV isolates in three broad bean cultivars were compared (Table 4). BYMV was detected by DAS-ELISA in all plants with mosaic symptoms, whereas it was not detected in plants without mosaic. The percentage of pathotype II transmission ranged from 0.0–4.2%, but the percentage of other pathotypes was 0.0–1.6%. Pathotype II tended to show a higher seed-transmissibility in broad bean than the other pathotypes.

DAS-ELISA with polyclonal antibodies

The results of DAS-ELISA performed with 14 BYMV isolates plus two CIYVV isolates and six antisera are shown in Table 5. None of the antisera gave perceptible reactions with crude extracts from noninoculated broad bean plants. Although all BYMV and CIYVV isolates except CIYVV-92-3, which did not react with the antiserum to BYMV-90-2, reacted with these six antisera to

BYMV or CIYVV, the reactivities among BYMV and CIYVV isolates were not identical. Two antisera to CIYVV reacted more strongly with the two CIYVV isolates than the 14 BYMV isolates. In contrast, all antisera to BYMV did not react more strongly with the 14 BYMV isolates than the two CIYVV isolates. BYMV-90-2, B, and S, which were included in pathotype II, reacted more strongly with the antiserum to BYMV-90-2 than any other isolates. However, these differences in reactivities do not seem to be essential for distinguishing serotypes.

TAS-ELISA with monoclonal antibodies

Using purified BYMV-90-2, CIYVV-90-1 and CIYVV-92-3, we obtained 16 MAbs which reacted with homologous virus in TAS-ELISA. The results of isotype analysis and the reactivity with purified virus and SDS-protein in TAS-ELISA and ACP-ELISA are shown in Table 6. Although MAb-2C4, -2D6, -3H8, and -6F8 either did not react or only weakly reacted with SDS-protein in TAS-ELISA and with purified virus and SDS-protein in ACP-ELISA, the other MAbs reacted with purified virus and SDS-protein in both ELISAs.

These MAbs were tested with 28 BYMV and two

Table 5. Reactivity of 14 bean yellow mosaic virus (BYMV) and two clover yellow vein virus (CIYVV) isolates in DAS-ELISA using six polyclonal antisera

Isolate	Pathotype	Polyclonal antisera to						
		BYMV-92-2	BYMV-90-2	BYMV-P	BYMV-O	CIYVV-90-1	CIYVV-92-3	
BYMV-	92-2	I	0.97 ^{a)}	0.33	0.39	0.17	0.29	0.12
	G1		0.60	0.45	0.40	0.33	0.31	0.69
	IbG		0.21	0.28	0.28	0.19	0.39	0.67
	A-2		0.55	0.22	0.24	0.20	0.20	0.32
	90-2	II	0.40	1.15	0.46	0.44	0.44	0.54
	B		0.25	0.91	0.37	0.37	0.32	0.22
	S		0.56	0.90	0.57	0.51	0.61	0.50
	P	III	0.15	0.20	0.66	0.22	0.19	0.11
	102		0.49	0.63	0.79	0.49	0.74	0.76
	CS		0.44	0.51	0.62	0.49	0.43	0.25
	O	IV	0.45	0.10	0.51	1.02	0.29	0.22
	92-1		0.07	0.33	0.30	0.30	0.58	0.49
G		0.16	0.41	0.49	0.24	0.15	0.44	
Gla		0.20	0.30	0.30	0.13	0.45	0.80	
CIYVV-	90-1		0.12	0.25	0.16	0.22	1.43	1.04
	92-3		0.17	0.03	0.21	0.22	0.82	1.45
Healthy (control)			0.00	0.02	0.01	0.00	0.02	0.01

a) Each value was the average of absorbance values at 405 nm from three experiments using three wells in each experiment.

Table 6. Characteristics and reactivities of monoclonal antibodies to BYMV-90-2, to CIYVV-90-1 and to CIYVV-92-3 in TAS-ELISA and ACP-ELISA

Monoclonal antibody	Isotype	TAS-ELISA ^{a)}		ACP-ELISA ^{a)}	
		Purified Virus	SDS-protein	Purified Virus	SDS-protein
BYMV-90-2	1F3	IgG ₁	+++ ^{b)}	+++	+++
	2B4	IgG ₁	++	++	++
	2C4	IgG ₃	++	—	—
	2C5	IgM	+	+	+
	3F9	IgG ₃	++	++	+++
	3F11	IgG ₃	++	+	++
	4G8	IgG ₃	++	++	++
	4H9	IgG ₃	++	++	++
CIYVV-90-1	1A2	IgG _{2a}	++	+++	+++
	1C8	IgG ₁	++	++	++
	2H8	IgG _{2a}	++	+++	+++
	3G9	IgG ₁	++	++	++
	5F2	IgG _{2a}	++	+++	+++
CIYVV-92-3	2D6	IgG ₁	+++	—	+
	3H8	IgG ₃	++	—	—
	6F8	IgG ₁	+++	—	+

a) Homologous virus was used for each antigen preparation of ELISA.

b) Serological reactivity, measured as the absorbance at 450 nm after 1 hr or 3 hr of substrate incubation: —, $A < 0.1$; +, $0.1 < A < 0.5$; ++, $0.5 < A < 1.5$; +++, $A > 1.5$.

CIYVV isolates (Table 7). Mab-1F3 gave medium to strong reactions with pathotypes I, II and III, and CIYVV-90-1. Mab-2C4 reacted strongly with only pathotype II. Mab-2B4, -2C5, -3F9, -3F11 -4G8 and -4H9 reacted with all BYMV isolates in pathotypes II and III, and with some BYMV isolates in pathotypes I and IV. In the case of MABs to CIYVV, Mab-5F2 was considered to be BYMV subgroup-specific since it gave medium to

strong reactions with all BYMV and CIYVV isolates tested and did not react with the other potyviruses (data not shown). Besides reacting strongly with the two CIYVV isolates, Mab-1A2 and -2H8 also showed a strong reaction with pathotype III.

Additional BYMV samples

Seventy-one BYMV-infected broad bean samples from a spring survey in 1990-91 of broad bean viruses on

Table 7. Reactivity of 28 bean yellow mosaic virus (BYMV) and two clover yellow vein virus (CIYVV) isolates in Japan by TAS-ELISA using 16 monoclonal antibodies

Isolate		Patho- type	Monoclonal antibody to									
			BYMV-90-2					CIYVV-90-1				CIYVV-92-3
			1F3	2C4	2C5	4G8	4H9 ^{a)}	1C8	2H8 ^{b)}	3G9	5F2	2D6 ^{c)}
BYMV-	92-2	I	+++ ^{d)}	—	++	++	—	—	—	+	++	—
	G1		+++	—	++	+++	+++	—	+	—	++	—
	IbG		++	—	+	++	—	—	—	—	++	—
	G2		++	—	+	++	+++	—	+	—	++	—
	Gla3		++	—	++	++	+++	—	+	—	++	—
	A-2		++	—	—	++	—	—	—	—	++	—
	Cro4		++	—	++	—	+	—	—	—	+	—
	90-2	II	+++	+++	++	+++	+++	—	—	++	++	—
	B		+++	+++	+	++	+++	—	—	+	++	—
	S		+++	+++	++	+++	+++	—	—	++	++	—
	90-3		+++	++	++	++	+++	—	—	++	++	—
	90-5		+++	+++	++	++	+++	—	—	++	++	—
	94-1		++	++	++	++	++	—	—	++	++	—
	94-2		++	+++	++	+++	+++	—	—	++	++	—
	MB4		+++	+++	++	+++	+++	—	—	++	++	—
	MB5	+++	+++	++	+++	+++	—	—	++	++	—	
	P	III	++	—	+	++	++	—	++	++	++	—
	102		+++	—	++	+++	+++	—	+++	+	+++	—
	CS		+++	—	++	+++	+++	—	+++	+++	+++	—
	O	IV	—	—	—	—	++	—	—	—	+	—
	92-1		—	—	++	+++	—	—	—	—	++	—
	G		—	—	++	—	+++	—	—	—	++	—
	Gla		—	—	+	++	++	—	—	—	++	—
	Gla4		—	—	+	++	++	—	—	—	++	—
	Fri		—	—	+	—	++	—	+	—	++	—
	121		—	—	++	+	++	—	+	++	++	—
	S-2		—	—	+	++	++	—	—	—	+	—
	B-1		—	—	+	—	++	—	—	—	++	—
CIYVV-	90-1	+++	—	—	—	—	+++	+++	+++	+++	—	
	92-3	—	—	—	—	—	—	+++	—	+++	+++	

a) The results obtained with monoclonal antibodies 2B4, 3F9 and 3F11 were similar.

b) The result obtained with a monoclonal antibody 1A2 was similar.

c) The results obtained with monoclonal antibodies 3H8 and 6F8 were similar.

d) Serological reactivity, measured as the absorbance at 450 nm after 1 hr or 3 hr of substrate incubation: -, $A < 0.1$; +, $0.1 < A < 0.5$; ++, $0.5 < A < 1.5$; +++, $A > 1.5$.

Shikoku island²⁶⁾ and seven BYMV-infected clover samples (provided by N. Iizuka) were examined by TAS-ELISA with MAb-2C4, -2H8 and -5F2. All 71 broad bean samples reacted with MAb-5F2, 69 of which reacted with MAb-2C4, which is specific to Pathotype II. All of the clover samples reacted with MAb-5F2. Five of these samples also reacted with MAb-2H8, meaning they are considered to be infected with pathotype III.

DISCUSSION

When 15 bean cultivars were inoculated with 28 BYMV isolates collected from different hosts and locations in Japan, the isolates could be clearly classified into four pathotypes (pathotype I-IV) on the basis of the reactions of the bean cultivars, Honkintoki, Kentucky Wonder, Master Piece, and Top Crop. These pathotypes

differed in both their infection of *N. benthamiana* and *N. clevelandii* and seed-transmissibility. Pathotypes I and IV systemically infected *N. benthamiana* and *N. clevelandii*, whereas pathotype III locally infected *N. clevelandii*. Pathotype II caused either local or no infections on *N. benthamiana* and *N. clevelandii*. Pathotype II had higher seed-transmissibility in broad bean than other pathotypes did.

Previously, Inouye¹²⁾ reported that BYMV in Japan could be classified into four strains (B, P, O and N strains) on the basis of host reaction. The B and P strains were characterized by poor pathogenicity to bean cultivars, compared with the O strain. The P strain was also reported to be characterized by bright yellow mosaic symptoms on pea compared to mosaic symptoms caused by the B strain. According to our classification, the B strain of BYMV in pathotype II, the P strain in

pathotype III and the O strain was in pathotype IV.

BYMV pathotypes seemed to be correlated to the original host from which the isolate was obtained. Nine out of 10 BYMV isolates from broad bean (90-2, B, S, 90-3, 90-5, 94-1, 94-2, MB4 and MB5) were in pathotype II. By TAS-ELISA using MABs to BYMV or CIYVV, 69 out of 71 BYMV-infected broad bean samples were considered to be infected with pathotype II. Randles *et al.*²¹⁾ reported that BYMV isolates from broad bean in South Australia showed different host reactions from those of other typical BYMV isolates. Fortass *et al.*⁸⁾ also reported that Dutch and Syrian BYMV isolates from broad bean differed from other BYMV isolates on the basis of reactions of pea, bean and broad bean. These results indicated that BYMV isolates from broad bean appeared to be especially adapted to broad bean, also confirming that pathotype II can be transmitted comparatively more easily through broad bean seeds than can the other pathotypes. Four out of seven BYMV isolates from gladiolus (G1, IbG, G2 and Gla3) were in pathotype I and three out of seven (G, Gla and Gla4) were in pathotype IV. In a field survey of gladiolus and broad bean in Kagoshima prefecture, all BYMV isolates from gladiolus were included in pathotype I or IV, whereas all BYMV isolates from broad bean were included in pathotype II (personal communication from H. Iwai of Faculty of Agriculture, Kagoshima University). Tsuchizaki *et al.*³³⁾ reported that four out of 14 BYMV isolates from red clover were included in the P strains (pathotype III) and 10 were included in the O strains (pathotype IV). In this study, two out of three BYMV isolates from red clover (102 and CS) were in pathotype III and one (92-1) was in pathotype IV. By TAS-ELISA using MABs to BYMV or CIYVV, five out of seven BYMV infected clover samples were considered to be infected with pathotype III. Although more BYMV isolates in Japan needed to be compared, pathotype I usually prevailed among gladiolus, pathotype II among broad bean, pathotype III among clover, and pathotype IV among gladiolus and clover.

At first, pea mosaic virus (PMV) was considered to differ from BYMV in the bright yellow mosaic symptoms on pea compared to the mild mosaic symptoms caused by BYMV, in its poor pathogenicity to bean cultivars and in its weak hybridization between PMV and BYMV RNAs using either randomly-primed cDNA or a cDNA probe corresponding to the 3'-noncoding region of BYMV^{2,22,32)}. Recently, PMV has been considered to be a strain of BYMV on the basis of a comparison of high-performance liquid chromatographic peptide profiles of PMV coat protein and sequence of the 3'-terminal region of PMV^{17,38)}, and was very closely related to BYMV-CS^{30,38)}. Inouye¹²⁾ pointed out PMV was synonymous with the P strain of BYMV on the basis of the bright yellow mosaic symptoms induced on pea and its poor pathogenicity to bean cultivars. Since both BYMV-P and BYMV-CS were in pathotype III, PMV (or pea mosaic strain of BYMV) was also consid-

ered to be included in pathotype III.

Our results demonstrated serological variability in Japanese isolates of BYMV, corresponding, to some degree, to biological variability. These data also indicated that there are risks in using a single MAB for the diagnosis of BYMV and CIYVV, and in using only one or two isolates of a given potyvirus to screen the reactivity of a particular MAB.

In TAS-ELISA, MAB-1F3 reacted with pathotypes I, II and III, and CIYVV-90-1. MAB-1A2 and -2H8 gave medium to strong reactions with two CIYVV isolates and pathotype III, showing CIYVV isolates have at least one common epitope with pathotype III that the other pathotypes did not have. MAB-3G9 reacted with CIYVV-90-1, BYMV-121, pathotypes II and III. MAB-5F2 reacted with all BYMV and CIYVV isolates in this study. These results confirmed the close relationship of BYMV with CIYVV^{1,2,14,15,24)}, and showed that there are at least four epitopes that are common in BYMV and CIYVV. In contrast, MAB-2B4, -2C5, -3F9, -3F11, -4G8 and -4H9 reacted with both pathotypes II and III, as well as some isolates in pathotypes I and IV, indicating serological differences in pathotypes I and IV. MAB-2C4 reacted with only pathotype II, indicating that pathotype II had at least one epitope that was common in pathotype II but was not detectable in other pathotypes and CIYVV isolates. Although MAB-1C8 reacted only with CIYVV-90-1, MAB-2D6, -3H8 and -6F8 reacted only with CIYVV-92-3. These results indicate that at least four epitopes differ between BYMV and CIYVV.

The N-terminal of the CP of potyviruses is surface-located and contains the virus-specific epitopes, whereas the core protein region of the CP is highly conserved between potyviruses and contains the virus-common cross-reactive epitopes²⁷⁻²⁹⁾. Although we did not localize the epitopes recognized by the virus-specific and the BYMV- and CIYVV-cross-reactive MABs, we would predict that the virus-specific MABs recognize the surface-located N-terminal epitopes, but the cross-reactive MABs recognize non-surface core protein epitopes.

In conclusion, CIYVV isolates in Japan could be grouped into four pathotypes on the basis of reactions of the bean cultivars, Honkintoki, Kentucky Wonder, Master Piece, and Top Crop. This biological variability corresponded, to some degree, to serological variability. This information should be useful for epidemiological studies, determining potential control strategies, breeding for resistance and developing reliable diagnostic techniques for BYMV.

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和 文 摘 要

笹谷孝英・野津祐三・小金澤碩城：日本に発生するインゲンマメ黄斑モザイクウイルス (BYMV) の病原性と血清学的性状の比較

日本の異なる地域および植物から分離したインゲンマメ黄斑モザイクウイルス (BYMV) 28 分離株について、判別植物の反応と血清反応を比較した。BYMV はインゲン 15 品種の反応で 4 つの Pathotype に分けることができた。Pathotype I はインゲン品種の本金時のみに全身感染を示し、他の品種には局部感染であった。Pathotype II は本金時、ケンタッキーワンダーおよび他 4 品種に全身感染を示し、Pathotype III は本金時、ケンタッキーワンダー、マスターピースおよび他 4 品種に全身感染を示し、Pathotype IV は今回用いた 15 品種すべてに全身感染を示した。Pathotype II に属する BYMV はソラマメにおいて他の Pathotype に属するものより高い種子伝染性を示した。BYMV あるいはクロウバ葉脈黄化ウイルス (CIYVV) に対する 16 種のモノクローナル抗体 (MAb) を用いた TAS-ELISA で、BYMV 28 分離株には血清学的差異が観察され、病原性とある程度一致したが、ポリクローナル抗体を用いた DAS-ELISA では、分離株間での顕著な差異は観察されなかった。MAb-1F3 は Pathotype I, II, III と CIYVV の 1 株と反応した。MAb-2C4 は Pathotype II のみと反応し、MAb-5F2 は今回用いた BYMV と CIYVV すべての株と反応した。MAb-2B4, -2C5, -3F9, -3F11, -4G8 および -4H9 は Pathotype II と III のすべてと、Pathotype I と IV の一部の株と反応した。MAb-1A2 と -2H8 は Pathotype III と CIYVV 2 分離株と強く反応した。以上より、日本の BYMV は病原性および血清学的に変異に富んでおり、4 つの Pathotype に分かれることが明らかとなった。