COMPARISON OF COAT PROTEIN EPITOPES OF TWO ZUCCHINI YELLOW MOSAIC VIRUS ISOLATES

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Summary. – Serological differences between two zucchini yellow mosaic virus (ZYMV) isolates (ZYMV-169 and ZYMV-M) obtained from two distinct geographical locations in Japan were determined by mapping epitopes on the coat proteins (CPs) of the two isolates. A total of 45 monoclonal antibodies (MAbs) against the two isolates were produced and the epitopes on the CPs were delineated by reacting these MAbs with trypsin-treated ZYMV particles and *Escherichia coli*-expressed ZYMV CP fragments. Six MAbs of groups I-a and I-b, specific for ZYMV-169, recognised two epitopes in the N-terminal region at amino acids (aa) 1–28 and 6–41 of ZYMV-169 CP. Fourteen MAbs of group II, specific for ZYMV-M, recognised epitopes in the N-terminal region of ZYMV-M CP. Twenty-one MAbs of groups III-a, III-b(i), III-b(ii), and III-b(iii), reacting with both isolates, recognised four epitopes; one epitope was located in the N-terminal region at aa 6–28 and the remaining three epitopes were located in the core region at aa 42–95, 171–227 and 228–259 of ZYMV CPs.

Key words: zucchini yellow mosaic virus; coat protein; monoclonal antibody; fusion protein; epitope

Introduction

ZYMV (genus *Potyvirus*, family *Potyviridae*) is responsible for major economic losses in cucurbit crops in many parts of the world (Lisa *et al.*, 1981). ZYMV is very efficiently transmitted from plant to plant by several aphid species in a non-persistent manner (Lisa and Lecoq, 1984). The virus particle is a flexible filament and contains a positive-sense single-stranded 9.5 kb RNA (Gal-On *et al.*, 1990). The potyvirus RNA is translated to a polyprotein precursor which is subsequently cleaved by virus-coded proteolytic enzymes to eight polypeptides (Dougherty and Carrington, 1988). The location of potyvirus CP gene is close to the 3'-end of the virus genome (Dougherty and Carrington, 1988), and the N-terminal region of the CP is known as one of the most variable parts of the potyvirus genome (Shukla *et al.*, 1991).

In Japan, a ZYMV isolate was first reported from Okinawa island (tropical region) by Ohtsu *et al.* (1985). Subsequently, other ZYMV isolates have been collected from four major islands (temperate region) of Japan. ZYMV-169 originating from Okinawa island was the most distinct isolate compared to other ZYMV isolates from the whole world when aa sequences of their CPs were analysed by phylogenetic tree (Kundu *et al.*, 1997). ZYMV-M from Kyushu island was very similar to many other isolates from the USA and Israel. ZYMV-169 and ZYMV-M did not show any differences in their host range but showed serological differences in double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using polyclonal antibodies (PAbs). Moreover, the aa sequence of ZYMV-169 CP showed 15 aa substitutions compared to that of ZYMV-M CP. Nine of these substitutions were located in the N-terminal

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Abbreviations: aa = amino acid; ALP = alkaline phosphatase; ATA-ELISA = antibody-trapped antigen ELISA; CP = coat protein; DAS-ELISA = double antibody sandwich ELISA; ELISA = enzyme-linked immunosorbent assay; FP = fusion protein; GST = glutathione S-transferase; i.v. = intravenous(ly); MAb = monoclonal antibody; nt PAb = polyclonal antibody, PBS = phosphate-buffered saline; PBS-T = PBS-Tween-20; PCR = polymerase chain reaction; PTA-ELISA = plate-trapped antigen ELISA; SDS = sodium dodecyl sulphate;WB = Western blot; ZYMV = zucchini yellow mosaic virus

region while the remaining six were in the core region (Kundu *et al.*, 1997).

MAbs to ZYMV-169 and ZYMV-M were produced and used to follow the prevalence of various biologically similar ZYMV isolates.

Materials and Methods

Virus isolates and their purification. ZYMV-169 and ZYMV-M (Kundu *et al.*, 1997) were propagated on *Cucurbita maxima* Duch. cv. Hokoseihi and purified according to Sako *et al.* (1980).

Trypsin treatment. Purified ZYMV particles were treated with trypsin (12.0 μg of trypsin per mg of virus) in 10 mmol/l Tris.HCl pH 8.0 at 37°C for 2.5 hrs. Then the treated virus particles were subjected to Western blot (WB) analysis (Towbin *et al.*,1979).

Expression of ZYMV CP in Escherichia coli. The pGEX3X vector (Pharmacia) was used to express a part of ZYMV CP with glutathione S-transferase (GST) in *E. coli*. A series of ZYMV CP gene fragments were amplified from ZYMV CP-cDNA clones (Kundu *et al.*, 1997) by polymerase chain reaction (PCR) (Saiki *et al.*, 1988). Synthetic oligonucleotide primers used in this study are listed in Table 1. The PCR-amplified CP gene fragments were digested with *Bam*HI and *Eco*RI, and cloned into pGEX3X vector. Then the recombinant plasmids were introduced into *E. coli* XL1-Blue. Expression of the recombinant plasmids was carried out as described previously (Ohshima *et al.*, 1994). Nucleotide sequences of the junction between GST and ZYMV CP, and that of ZYMV CP gene were determined by using Dye Terminator Cycle Sequencing Kit (Abi).

Synthetic peptide SGTQPTVADAGATK from the N-terminal region of ZYMV-M CP was synthesised by the "Fmoc" solid-phase method (Van Regenmortel *et al.*, 1988). The C-terminus of the peptide was conjugated to bovine serum albumin by ε-maleimidocaproic acid N-hydroxysuccinimide ester using an additional cysteine added to the C-terminal end of the peptide (Van Regenmortel *et al.*, 1988).

Polyclonal antibodies (PAbs). A rabbit was immunised by three intramuscular injections of a total of 3.0 mg of purified ZYMV emulsified with Freund's complete adjuvant (first injection) or Freund's incomplete adjuvant (second and third injections) at two-week intervals. The booster was given by intravenous (i.v.) injection of 1.5 mg of purified ZYMV 10 days before bleeding. DAS-ELISA (Clark and Adams, 1977) using PAbs was performed to investigate the reactivities of the two ZYMV isolates. Microplates (Nunc) were coated with 2.0 μg/ml ZYMV-specific anti-rabbit IgG in carbonate buffer pH 9.6 at 37°C for 4 hrs. After washing with phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PBS-T), the plates were incubated with serial tenfold dilutions (10.0 μg/ml – 0.001 μg/ml) of purified ZYMV-169 and ZYMV-M in PBS and then with alkaline phosphatase (ALP)-labelled rabbit anti-ZYMV IgG conjugate diluted in PBS-T at the ratio of 1:1000. Finally, *p*-nitrophenyl phosphate (1.0 mg/ml) was added to react at 25°C for 1 hr.

A_{405} was measured using an Immuno Mini NJ-2300 ELISA reader. Titres of anti-ZYMV IgGs were determined by DAS-ELISA as described above with two modifications. Serial tenfold dilutions (10.0 μg/ml – 0.001 μg/ml) instead of the concentration of 2 μg/ml of anti-ZYMV IgGs were used. Purified ZYMV-169 and ZYMV-M were used at the concentration of 2.0 μg/ml.

Monoclonal antibodies (MAbs). Six-week-old female BALB/c mice were immunised by three intraperitoneal injections of a total of 100 μg of purified ZYMV emulsified with Freund's complete adjuvant (first injection) or Freund's incomplete adjuvant (second and third injections) at two-week intervals. The booster was given by i.v. injection of 50 μg of purified ZYMV in the tail. Three days after the booster, spleen cells were fused with myeloma cells of the line P3-NS1/1-Ag4-1. Fused cells were cultured in a selective HAT medium (hypoxanthine, aminopterin and

Table 1. Synthetic oligonucleotide primers used for the production of FPs containing complete CPs or CP fragments of ZYMV-169 and ZYMV-M

List of oligonucleotide primers			
No. of primer	nt position[a]	Virus isolate	Direction of primer
1	133–163	ZYMV-169	Forward
2	238–255	ZYMV-169	Reverse
3	133–163	ZYMV-M	Forward
4	238–255	ZYMV-M	Reverse
5	949–969	Both isolates	Reverse
6	256–275	Both isolates	Forward
7	396–417	Both isolates	Reverse
8	504–522	Both isolates	Reverse
9	624–642	Both isolates	Reverse
10	796–813	Both isolates	Reverse
11	133–148	ZYMV-169	Forward
12	133–148	ZYMV-169	Reverse
13	148–164	Both isolates	Forward
14	196–216	Both isolates	Reverse
15	217–234	ZYMV-169	Forward

List of FPs and their respective primer pairs		
FPs	aa position	Primer pair
ZYMV-169 CP (aa 1-41)	1-41	1, 2
ZYMV-M CP (aa 1-41)	1-41	3, 4
ZYMV-169 CP (aa 1-28)	1-28	1, 14
ZYMVM CP (aa 1-28)	1-28	3, 14
ZYMV-169 CP (aa 1-5)	1-5	11, 12
ZYMV-169 CP (aa 6-28)	6-28	13, 14
ZYMV-M CP (aa 6-28)	6-28	13, 14
ZYMV-169 CP (aa 6-41)	6-41	13, 2
ZYMV-M CP (aa 6-41)	6-41	13, 4
ZYMV-169 CP (aa 29-41)	29-41	15, 2
ZYMV-169 CP	1-279	1, 5
ZYMV-M CP	1-279	3, 5
ZYMV-169 CP (aa 42-279)	42-279	6, 5
ZYMV-M CP (aa 42-279)	42-279	6, 5
ZYMV-M CP (aa 42-227)	42-227	6, 10
ZYMV-M CP (aa 42-170)	42-170	6, 9
ZYMV-M CP (aa 42-130)	42-130	6, 8
ZYMV-M CP (aa 42-95)	42-95	6, 7

[a]Based on the nucleotide sequences of CPs of ZYMV-169 and ZYMV-M (Kundu *et al.*, 1997).

Table 2. Characteristic of MAbs against ZYMV-169 and ZYMV-M

Groups	MAbs	Types of epitopes[a]	Reactions to ZYMV isolates[b]			
			ATA-ELISA		PTA-ELISA	
			ZYMV-169	ZYMV-M	ZYMV-169	ZYMV-M
I	169–43, 169–44, 169–45, 169–48, 169–50, 169–59	Metatope	+	–	+	–
II	M-6, M-12, M-13, M-16, M-18, M-19, M-22, M-24, M-27, M-29, M-31, M-32, M-33, M-34	Metatope	–	+	–	+
III	169–42, 169–46, 169–47, 169–49, 169–55, 169–58, M-9, M-17, M-28, M-30, M-8, 169–52, 169–53, 169–54, 169–56, 169–57, M-10, M-11, M-14, M-15, M-20	Metatope	+	+	+	+
IV	169–41, 169–62, M-25, M-26	Neotope	+	+	–	–

[a]Based on reactivities of MAbs in ELISA and WB analysis.
[b]Purified ZYMV (2 µg/ml) was used in the reaction with MAb. A_{405} was measured after the 1-hr-reaction with substrate. (–) = A_{405} <0.1, (+) = 0.1≤A_{405}<1.4.

thymidine in RPMI-1640). After 9 days, supernatants from wells containing hybridomas were screened for antibody production by two indirect ELISA procedures according to Massalski and Harrison (1987) with some modifications. (1) Plate-trapped antigen ELISA (PTA-ELISA): plates were coated with 2.0 µg/ml purified ZYMV in carbonate buffer pH 9.6 at 4°C for 14 hrs. Then the plates were washed three times with PBS-T. After 1 hr of incubation with 1% non-fat dried milk in PBS-T at 37°C, the plates were incubated with a culture supernatant for 4 hrs. Then ALP-labelled goat anti-mouse IgG was added to the plates and the reaction was visualised with *p*-nitrophenyl phosphate. (2) Antibody-trapped antigen ELISA (ATA-ELISA): plates were coated with 2.0 µg/ml rabbit anti-ZYMV IgG in carbonate buffer pH 9.6. Then 2.0 µg/ml purified ZYMV in PBS was added. The subsequent steps were the same as those in PTA-ELISA. Hybridoma cells secreting MAbs to ZYMV were cloned by the limiting dilution method (Jordan, 1990). Isotyping of MAbs was performed using a Mouse Typer Sub-isotyping Kit (Bio-Rad).

Western blot (WB) analysis. Purified ZYMV particles, trypsin-treated ZYMV particles, fusion proteins (FPs), and the synthetic peptide were dissociated by the addition of an equal volume of the sample buffer (0.0625 mol/l Tris.HCl pH 6.8, 2% sodium dodecyl sulphate (SDS), 5% 2-mercaptoethanol, 10% glycerol, and 0.001% bromophenol blue) (Laemmli, 1970) and then heated for 3 mins in boiling water. They were electrophorsed on 12.5% polyacrylamide gels in the presence of SDS (Laemmli,1970) using a Bio-Rad apparatus, model Mini-Protean II. After electrophoresis, the gels were either stained with Coomassie Brilliant Blue or subjected to WB analysis (Towbin *et al.*, 1979). In the latter case, the gels were blotted to Immobilon polyvinyl difluoride membranes (Millipore) and reacted with antibodies. Horseradish peroxidase-labelled goat anti-rabbit (for PAb) or goat anti-mouse (for MAb) IgG was used as secondary antibody. The Konica Immunostaining HPR-1000 kit was used for staining.

Results

Reactivities of PAbs in DAS-ELISA

Reactivities of PAbs specific for ZYMV-169 and ZYMV-M were determined using homologous and heterologous antigens in DAS-ELISA (data not shown). When the IgG and conjugate specific for ZYMV-169 were used, 10 µg/ml ZYMV-169 and ZYMV-M showed A_{405} values of 2.04 and 0.79, respectively. On the other hand, 10 µg/ml ZYMV-M and ZYMV-169 showed A_{405} values of 2.05 and 0.82, respectively, when the IgG and conjugate specific for ZYMV-M were used. Both anti-ZYMV IgGs had homologous titres of 1/1000 and heterologous titres of 1/100 in DAS-ELISA.

Characterisation of MAbs

Forty-five stable hybridoma secreting MAbs against the two isolates of ZYMV were obtained from six fusion experiments with ZYMV-169 and ten fusion experiments with ZYMV-M. Nineteen MAbs against ZYMV-169 and 26 MAbs against ZYMV-M were produced. The MAbs against ZYMV-169 were designated 169–41 to 169–69, and MAbs against ZYMV-M were designated M-1 to M-34. The isotypes of the MAbs were IgG1 (M-6, M-8, M-12, M-17, M-27, M-28, M-31, M-32, M-33, and 169-56), IgG2a (M-10, M-11, M-24, M-25, M-26, M-29, M-30, M-34, 169–50, 169–52, 169–53, 169–54, 169–55, and 169–57), IgG2b (M-14, M-20, 169–59, and 169–6 2), and IgG3 (M-9, M-13, M-15, M-16, M-18, M-19, M-22, 169–41, 169–42, 169–43, 169–44, 169–45, 169–46, 169–47, 169–48, 169–49, and 169–58). As shown in Table 2, four MAbs (169–41, 169–62, M-25 and M-26) reacted with ZYMV only in ATA-ELISA. On

Table 3. Reactivities of MAbs to ZYMV CPs and trypsin-resistant ZYMV CP core regions in WB analysis

Groups	MAbs	CPs		Trypsin-resistant CP core regions	
		ZYMV-169	ZYMV-M	ZYMV-169	ZYMV-M
I	169–43, 169–44, 169–45,	+	–	–	–
II	M-6, M-12, M-13, M-16, M-18, M-19, M-22, M-24, M-27, M-29, M-31, M-32, M-33, M-34	–	+	–	–
III-a	169–42, 169–46, 169–47, 169–49, 169–55, 169-58, M-9, M-17, M-28, M-30,	+	+	–	–
III-b	169–52, 169–53, 169–54, 169–56, 169–57, M-8, M-10, M-20, M-11, M-14, M-15	+	+	+	+
IV	169–41, 169–62, M-25, M-26	–	–	–	–

Positive (+) and negative (–) reactions.

the other hand, the remaining 41 MAbs reacted with ZYMV in both ATA-ELISA and PTA-ELISA. The particles of ZYMV were presumably dissociated when diluted in the carbonate buffer in PTA-ELISA. The first four MAbs probably recognised neotopes on virus particles while the other 41 MAbs probably recognised metatopes present on virions and dissociated proteins (Van Regenmortel, 1982). These results were also confirmed by using protein subunits (data not shown). Out of the 45 MAbs six were specific for ZYMV-169, whereas 14 were specific for ZYMV-M. The remaining 25 MAbs reacted with both isolates.

Reactivities of MAbs to CPs and trypsin-resistant CP core regions in WB analysis

The trypsin treatment of a potyvirus removes the N- and C-terminal regions of CP, leaving a fully assembled virus particle composed of CP core region (Shukla *et al.*, 1988). The reactivities of the 45 MAbs to ZYMV CPs and trypsin-resistant ZYMV CP core regions in WB analysis divided the MAbs into five groups (Table 3). Six MAbs specific for ZYMV-169 of group I reacted with intact ZYMV-169 CP but not with ZYMV-169 CP core region. Since the aa sequences of C-terminal regions of both isolates were identical (Kundu *et al.*, 1997), MAbs of group I presumably recognised the N-terminal region of ZYMV-169 CP. Fourteen MAbs specific for ZYMV-M of group II reacted with intact ZYMV-M CP but not with ZYMV-M CP core region, indicating that MAbs of group II presumably recognised the N-terminal region of ZYMV-M CP. Ten MAbs of group III-a reacted with intact ZYMV CPs but not with ZYMV CP core region, indicating that these MAbs recognised the N- or C-terminal region of ZYMV CPs. Eleven MAbs of group III-b reacted not only with intact ZYMV CPs but also with ZYMV CP core region, indicating that these MAbs recognised ZYMV CP core region. A total of 41 MAbs of groups I, II, III-a and III-b reacted with ZYMV CPs in WB analysis, confirming that these MAbs recognised metatopes. However, four MAbs of group IV did not react with intact ZYMV CPs and trypsin-resistant ZYMV CP core regions in WB analysis, indicating that these MAbs recognised neotopes.

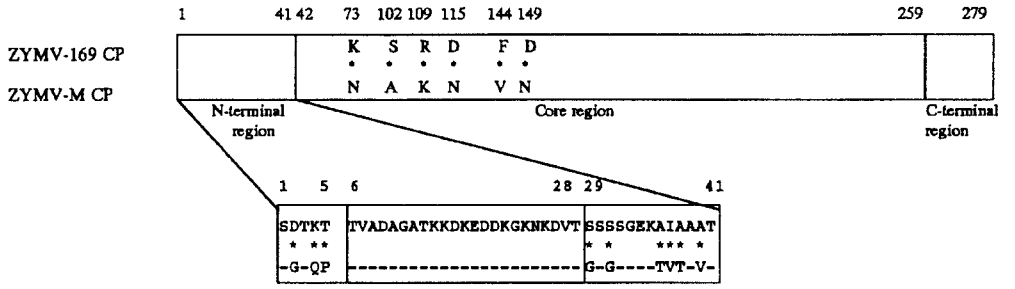
Production of FPs

A series of ZYMV CP fragments were expressed in *E. coli* to determine the location of epitopes on the CP. A total of 18 FPs (Table 1) were produced. All of these FPs except two (ZYMV-169 CP (aa 1-5) and ZYMV-169 CP (aa 29-41)) reacted with the respective ZYMV PAbs in WB analysis. Moreover, these FPs reacted strongly in WB analysis with all the MAbs except those of groups II and IV (Fig. 1). No MAbs reacted with the carrier protein, GST. The nucleotide sequences of the junctions between GST and ZYMV CP genes were determined and the nucleotide sequences of these recombinant clones were found identical to those of ZYMV-169 and ZYMV-M CP genes (data not shown).

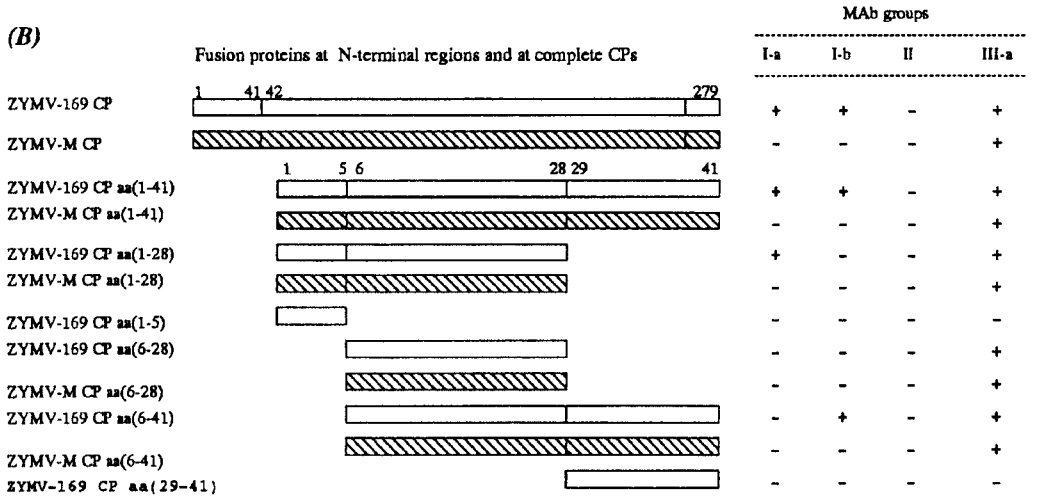
Fig. 1
Scheme of reactivities of MAbs to ZYMV CPs and CP fragments expressed in *E. coli* as FPs

(A) aa substitutions between ZYMV-169 and ZYMV-M CPs in the core and N-terminal regions (Kundu *et al.*, 1997). Dashes show no substitutions. (B) Reactivities of MAbs of groups I-a, I-b, II and III-a to FPs in the N-terminal region of ZYMV CPs. (C) Reactivities of MAbs of groups III-b(i), III-b(ii), III-b(iii) and IV to FPs in the core and C-terminal regions of ZYMV CPs. Positive (+) and negative (–) reactions in WB analysis (D) Summary of epitope locations on the CPs of ZYMV isolates based on the use of MAbs

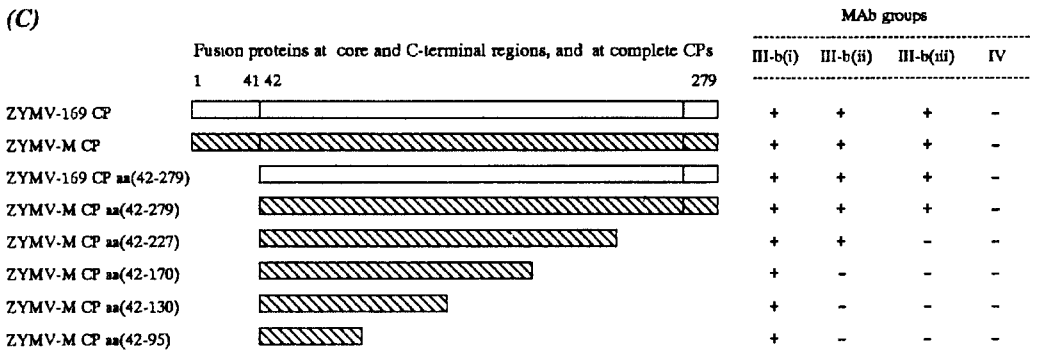
(A)



(B)



(C)



(D)

Groups	MAbs	Epitopes
I-a	169-45, 169-48, 169-50, 169-59	aa 1-28 of ZYMV-169 CP
I-b	169-43, 169-44	aa 6-41 of ZYMV-169 CP
II	M-6, M-12, M-13, M-16, M-18, M-19, M-22, M-24, M-27, M-29, M-31, M-32, M-33, M-34	N-terminal region of ZYMV-M CP
III-a	169-42, 169-46, 169-47, 169-49, 169-55, 169-58, M-9, M-17, M-28, M-30,	aa 6-28 of ZYMV CPs
III-b(i)	M-8	aa 42-95 of ZYMV CPs
III-b(ii)	169-56, 169-57, M-10, M-20	aa 171-227 of ZYMV CPs
III-b(iii)	169-52, 169-53, 169-54, M-11, M-14, M-15	aa 228-259 of ZYMV CPs
IV	169-41, 169-62, M-25, M-26	Not determined.

Reactivities of MAbs to N-terminal region

The reactivities of MAbs to N-terminal region of ZYMV CPs are shown in Fig. 1B. Four MAbs (169–45, 169–48, 169–50 and 169–59) of group I (designated group I-a) reacted with ZYMV-169 CP (aa 1-41) and ZYMV-169 CP (aa 1-28) but not with ZYMV-M CP (aa 1-41), ZYMV-M CP (aa 1-28), ZYMV-169 CP (aa 1-5), ZYMV-169 CP (aa 6-28), ZYMV-169 CP (aa 6-41) and ZYMV-169 CP (aa 29-41), indicating that these MAbs recognised an epitope between aa 1 and 28 of the N-terminal region of ZYMV-169 CP. The remaining two MAbs (169–43 and 169–44) of group I (designated group I-b) reacted with ZYMV-169 CP (aa 1-41) and ZYMV-169 CP (aa 6-41) but not with ZYMV-M CP (aa 1-41), ZYMV-M CP (aa 6-41), ZYMV-169 CP (aa 1-28), ZYMV-169 CP (aa 1-5), ZYMV-169 CP (aa 6-28) and ZYMV-169 CP (aa 29-41), indicating that these MAbs recognised an epitope between aa 6 and 41 of the N-terminal region of ZYMV-169 CP. However, 14 MAbs against ZYMV-M of group II did not react with any of the FPs from the N-terminal region of ZYMV-M CP and the FP containing complete ZYMV-M CP. Ten MAbs of group III-a reacted with ZYMV-169 CP (aa 6-28) and ZYMV-M CP (aa 6-28) from the N-terminal region; therefore, these MAbs recognised epitope(s) between aa 6 and 28 of the N-terminal region of ZYMV CPs.

Reactivities of MAbs to core and C-terminal regions

The reactivities of MAbs to core and C-terminal regions of ZYMV CPs are shown in Fig. 1C. A MAb (M-8, reacting with both isolates) of group III-b (designated group III-b(i)) reacted with ZYMV-M CP (aa 42-95), indicating that the MAb recognised an epitope between aa 42 and 95 of ZYMV CPs. On the other hand, four MAbs (169–56, 169–57, M-10 and M-20, reacting with both isolates) of group III-b (designated group III-b(ii)) reacted with ZYMV-M CP (aa 42-227) but not with ZYMV-M CP (aa 42-170), indicating that these MAbs recognised epitope(s) between aa 171 and 227 of ZYMV CPs. The remaining six MAbs (M-11, M-14, M-15, 169–52, 169–53 and 169-54, reacting with both isolates) of group III-b (designated group III-b(iii)) reacted with ZYMV-M CP (aa 42-279) but not with ZYMV-M CP (aa 42-227), indicating that these MAbs recognised epitope(s) between aa 228 and 279 of ZYMV CPs. Since the MAbs of group III-b(iii) reacted with the trypsin-resistant ZYMV CP core region (aa 42-259) (Table 3), the epitope was located between aa 228 and 259. MAbs of group IV (M-25, M-26, 169–41 and 169–63, reacting with both isolates) did not react with any of the FPs in WB analysis.

Discussion

Forty-five MAbs against the two isolates of ZYMV were produced using an identical procedure and used to map epitopes on the CPs of the two isolates. All the MAbs except four MAbs of group IV reacted with the respective ZYMV CP in WB analysis. Thus, the epitopes recognised by the MAbs were largely dependent on primary structure (Pappu *et al.*, 1995). Four MAbs of group I-a and two MAbs of group I-b, specific for ZYMV-169, reacted with the fragments aa 1-28 and aa 6-41 of the N-terminal region of ZYMV-169 CP. On the other hand, 14 MAbs of group II, specific for ZYMV-M, reacted in WB analysis with purified ZYMV-M but not with any of the FPs containing ZYMV-M CP. The non-reactivity of the MAbs of group II with the FPs was probably due to the fact that the fusion of a carrier (GST) with ZYMV-M CP inhibited the MAb binding. Unfortunately, these MAbs did not react with the synthetic peptide SGTQPTVADAGATK corresponding to a portion at the N-terminal region of ZYMV-M CP. Therefore, the MAbs of group II probably recognised a larger epitope than the 14 aa long region. The nine aa substitutions at the N-terminal region in the two isolates could influence the epitope formation in the N-terminal region.

Ten MAbs of group III-a, reacting with both isolates, recognised epitope(s) between aa 6 and 28 (T^{6}VADAGATKKDKEDDKGKNKDVT^{28}) of the N-terminal region of ZYMV CPs. Amino acid sequences of this portion were identical in both isolates. According to Shukla *et al.* (1988), the N-terminal region of CP of potyviruses is surface-exposed and is most immunodominant. Therefore, one of the immunodominant epitopes is located between aa 6 and 28 of the N-terminal region of ZYMV CPs and this region probably plays an important role in the cross-reactivity between ZYMV-169 and ZYMV-M in DAS-ELISA. On the other hand, 11 MAbs of groups III-b(i), III-b(ii) and III-b(iii), reacting with both isolates, recognised at least three epitopes in the core region. We cannot exclude the possible role of these epitopes on the core region of ZYMV CPs in the cross-reactivity between ZYMV-169 and ZYMV-M in DAS-ELISA. According to Shukla *et al.* (1989), the cross-reacting MAbs or PAbs recognise epitopes in the conserved core region of potyvirus CP. In addition to seven different epitopes, we found at least one neotope (recognised by four cross-reacting MAbs of group IV) on ZYMV CPs.

The results of this study revealed that at least seven epitopes were located on the ZYMV CPs. Out of the seven epitopes three were specific for ZYMV-169 or ZYMV-M. Thus, the specific MAbs can be used as molecular protein probes for investigating the prevalence of ZYMV in Japan.

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