

## NUCLEOTIDE SEQUENCES OF THE CYLINDRICAL INCLUSION PROTEIN GENES OF TWO JAPANESE ZUCCHINI YELLOW MOSAIC VIRUS ISOLATES

A. K. KUNDU<sup>1</sup>, K. OHSHIMA<sup>1\*</sup>, N. SAKO<sup>2</sup>, H. YAEGASHI<sup>3</sup>

<sup>1</sup>Laboratory of Plant Virology and <sup>3</sup>Laboratory of Plant Pathology, Faculty of Agriculture, Saga University, 1-banchi, Honjo-machi, Saga 840-8502, Japan; <sup>2</sup>Saga University, Saga, Japan

*Received January 26, 1999; accepted January 29, 1999*

**Summary.** – The nucleotide sequences of the cylindrical inclusion protein (CIP) genes of two Japanese zucchini yellow mosaic virus (ZYMV) isolates (ZYMV-169 and ZYMV-M) were determined. The CIP genes of both isolates comprised 1902 nucleotides and encoded 634 amino acids containing consensus nucleotide binding motif. The sequence similarities between the two isolates at the nucleotide and amino acid levels were 91% and 98%, respectively. When the CIP gene sequences of the Japanese ZYMV isolates were compared with those of previously reported ZYMV isolates, the nucleotide and amino acid sequence similarities ranged between 81% and 97%, and between 95% and 97%, respectively. Phylogenetic analysis of the deduced amino acid sequences of the CIP genes indicated that the Japanese ZYMV isolates were closely related to those of other ZYMV isolates.

**Key words:** zucchini yellow mosaic virus; Japanese isolates; cylindrical inclusion protein gene; phylogenetic analysis

ZYMV (*Potyvirus* genus, *Potyviridae* family) causes serious losses of cucurbitaceous crops worldwide (Lisa and Lecoq, 1984). Potyviral virion particles are composed of a single-stranded, positive-sense RNA of nearly 10 kb encapsidated by approximately 2000 copies of coat protein (CP) monomers in a helical fashion (Lindbo and Dougherty, 1994). The potyviral genome is covalently bound at the 5'-end to a virus-coded protein designated VPg (Hari, 1981) and has a poly(A) sequence at the 3'-end (Hari *et al.*, 1979). The genome contains a single open reading frame (ORF) that is translated into a large polyprotein and processed co- and post-translationally into eight or more proteins by three virus-coded proteinases (Dougherty and Semler, 1993). Out of these proteins, VPg and CP are the only gene products detected in

virus particles. Other gene products, P1-Pro, HC-Pro, P3, CIP, NIa, and NIb but not 6K1 and 6K2 have been detected in infected plants and characterized (Dougherty and Carrington, 1988; Rodríguez-Cerezo and Shaw, 1991).

All potyviruses induce the formation of characteristic „pinwheel,, cylindrical inclusions in the cytoplasm of infected cells (Edwardson, 1974), and this property has been considered one of the most important phenotypic criterion for assigning viruses to the *Potyviridae* family (Shukla *et al.*, 1989). Moreover, the relatively large size of CIP allows for easy detection and is potentially more useful than CP for diagnosis and classification of potyviruses (Yeh and Gonsalves, 1984). The CIP of potyviruses has been shown to be an RNA helicase (Lain *et al.*, 1989), and has a nucleic acid-stimulated ATPase activity (Lain *et al.*, 1991).

Recently, the nucleotide and deduced amino acid sequences of the CP genes of two Japanese ZYMV isolates have been compared with those of previously reported ZYMV isolates (Kundu *et al.*, 1997). In the present study, we have determined the nucleotide and deduced amino acid sequences of the CIP genes of the two Japanese ZYMV iso-

\*Corresponding author.

**Abbreviations:** aa = amino acid; CIP = cylindrical inclusion protein; CP = coat protein; ds = double-stranded; MLV = murine leukemia virus; nt = nucleotide; ORF = open reading frame; RT-PCR = reverse transcription-polymerase chain reaction; ZYMV = zucchini yellow mosaic virus

A N K A D E N E R T L M H M Y H I F S K K Q D D A P I Y N D 30  
 169 GCAAATAAAGCTGATGAAAAATGAAAGGACGTTAATGCACATGTATCACATTTTCAGCAAGAAACAGGATGATGCCACCATATACAATGAC 90  
 M -----T-----C-----G-----

F L E H V R N V R P D L E E T L L Y M A G A E V V A T Q A K 60  
 169 TTTCTTGAACATGTGCGCAATGTGAGACCAGATCTTGAGGAAACCTTATTGTACATGGCTGGCGCAGAAGTTGTTGCAACACAAGCGAAG 180  
 M -----T-----C-C-----T-----G-G-T-----

S A V Q I Q F E K I I A V L A L L T M C F D A E R S D A I F 90  
 169 TCAGCAGTCCAGATTCACTTCGAGAAAATTATAGCCGTGTTGGCGCTGCTCACTATGTGTTTGGACGCTGAAAGAAGTGACGCCATTTTC 270  
 M -----G-T-----A-----T-----C-----C-----T-----

K I L T K L K T V F G T V G E T V R L Q G L E D I E S L E D 120  
 169 AAGATTTTGACAAACGCTCAAAACGGTTTTTGGCAGGTTGGAGAAACGGTCCGGCTTCAAGGACTTGAGGACATTGAGAGCTTTGGAGGAC 360  
 M -----A-----A-----A-----A-----A-----

D K R L T I D F D I N T N D A Q S S T T F D V H F D D W W N 150  
 169 GACAAAGAGCTCACAAATTGACTTTGATATCAACACGAATGATGCTCAATCATCGACGACATTGATGTCATTTTGACGATTGGTGGAAAC 450  
 M --T-----T-----T--T-----C-G-----G-A--A-----T--C-----T

R Q L Q Q N R T V P H Y R T T G K F L E F T R N T A A F V A 180  
 169 CGACAGCTACAGCAAAATCGACAGTTCACATTACAGGACCACAGTAAATTCCTTGAATTTACAGAAACACTGCAGCTTTTGTGGCT 540  
 M --G-A-----T-----C-----T-----C-----

N E I A S S S E G E F L V R G A V G S G K S T S L P A H L A 210  
 169 AATGAAATAGCATCATCAAGTGAAGGAGAATTTTAGTTAGGGGAGCAGTGGGTTCTGAAAAATCAACAGAGCTTACCCGCCCATCTTGCT 630  
 M -----G-----G-C-----A-----A-----T-----T-A-----C

K K G K V L L L E P T R P L A E N V S R Q L A G D P F F Q N 240  
 169 AAGAGGGCAAGTTTTACTACTCGAGCTACACGCCCATTTGGCGGAGAATGTCAAGTAGGCAGTGGCGGGCGCATCTTTCTTTCAAAC 720  
 M -----T-----G-----A-----C-T-----A-----T--C--A-----A--A--T-----

V T L R M R G L S C F G S S N I T V M T S G F A F H Y Y V N 270  
 169 GTCACACTTAGAATGAGAGGGCTAAGTTGTTTTGGTTCAAGCAATATTACAGTGATGACGAGTGGTTTTGCTTTTCATTACTATGTCAAC 810  
 M --T-----C-----T-----C-----A-----A-----C-----T-----

N P H Q L M E F D F V I I D E C H V T D S A T I A F N C A L 300  
 169 AATCCACATCAATTAATGGAATTTGACTTCGTTATCATAGACGAATGTATGTCACGGACAGTGGCAGCATAGCCCTTCAATTGCGCACTC 900  
 M -----T--C-----C-----A-----A-----T-----

K E Y N F A G K L I K V S A T P P G R E C D F D T Q F A V X 330  
 169 AAAGAGTATAATTTTCTGCTGTAATGATTAAGTGTCTGCAACGCCCGCAGGACGAGAGTGGGATTTTGATACGCAATTCGCGGTGAAA 990  
 M -----C--C-----GA-----C-----

V K T E D H L S F Q A F V G A Q K T G S N A D M V Q H G N N 360  
 169 GTCAAAACGGAGGATCACCTTTCAATTCAGGCATTTGTTGGCGCTCAGAAGACTGGTTCAAATGCTGATATGGTTCAAGCATGGTAATAAC 1080  
 M -----A-----C--T-----C--T-----C-----A-----C-----

I L V Y V A S Y N E V D M L S K L L T E R Q F S V T K V D G 390  
 169 ATACTTGTGTATGTTGCAAGTTACAAACGAAGTGGACATGCTTCCAAGTTACTCACTGAGCGACAATTTTCAGTGACGAAGGTGGATGGA 1170  
 M -----C-----T--T--A-----A-----G

R T M Q L G K T T I E T H G T S Q K P H F I V A T N I I E N 420  
 169 CGAACATGCAACTTGGAAAAACCACTTGAACGCATGGAAGTACGAGGAGCCACATTTTCATAGTAGCCACAACATTATCGAAAAAT 1260  
 M -----G-----T-----A-----C-----C-----G-----

G V T L D V E C V V D F G L K V V A E L D S E N R C V R Y N 450  
 169 GGAGTGACGTTGGATGTTGAGTGTGTTGTTGATTTTGAGTTAAAGTGGTCCCGAGTTGGACAGTGAAAAATCGATGTGCGCTACAAAC 1350  
 M -----C-----A--A-----C-----G-----T-----

K K P V S Y G E R I Q R L G R V G R S K P G T A L R I G Y T 480  
 169 AAGAAACAGTTAGTTACGAGAAAGAAATTCAGCGCTAGGGAGAGTGGGAAGATCCAAGCTTGGAACTGCATTGCGGATAGGATACACA 1440  
 M -----T-----G--G-----C-----G-----T-----T-----GC-----

E K G I E S I S E F I A T E A A A L S F A Y G L P V T T H G 510  
 169 GAAAAAGGCATCGAGAGCATTTCTGAATTCATTGCAACAGAGGACGAGCCCTATCATTGTCATATGGGCTTCCAGTCACCACGCATGGG 1530  
 M -----C-----C-----C-----A-----T-----A-----A

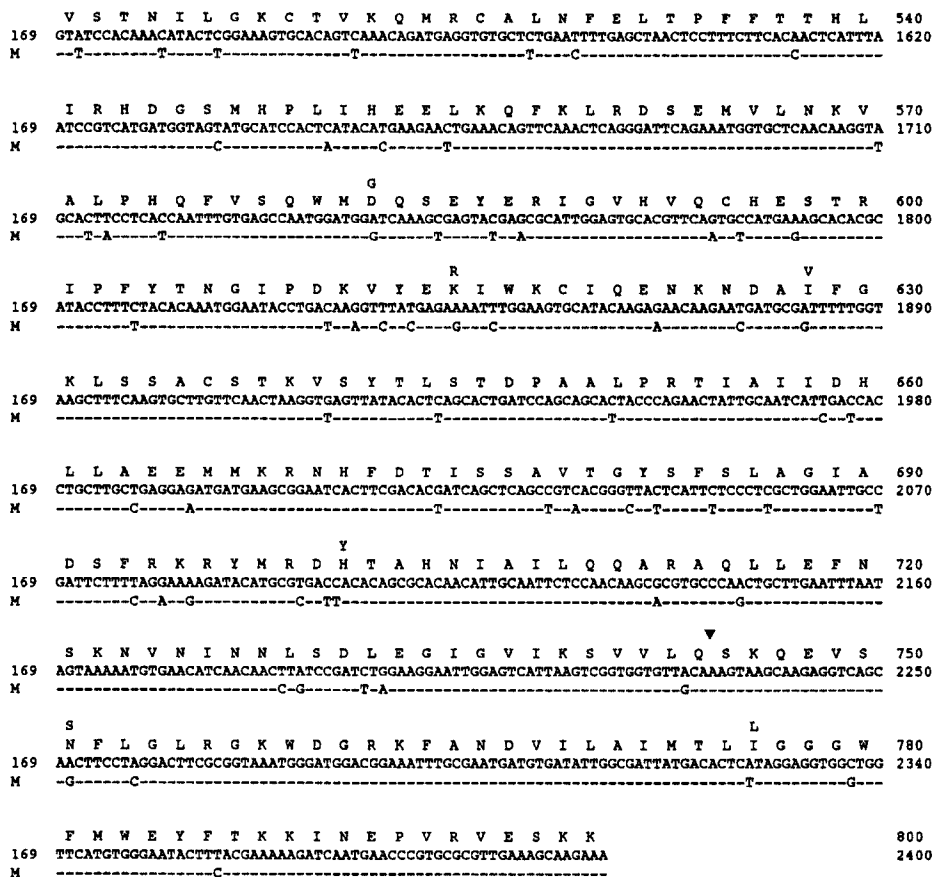


Fig. 1

#### Comparison of nucleotide and deduced amino acid sequences of CIP genes of ZYMV-169 and ZYMV-M cDNAs

Dashes show identical nucleotides in comparison to ZYMV-169. The deduced amino acid sequence of ZYMV-169 is shown above its nucleotide sequence. The different amino acids found in ZYMV-M are shown above the amino acids of ZYMV-169. The putative cleavage sites located between 6K1 and CIP, and between CIP and 6K2 genes are denoted by empty and full triangles, respectively.

lates and compared them with those of other reported ZYMV isolates by phylogenetic analysis.

The two Japanese ZYMV isolates (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). The genomic RNAs of the ZYMV isolates were extracted by the procedure described by Rosner *et al.* (1983). The reverse transcription-polymerase chain reaction (RT-PCR) (Ohshima *et al.*, 1994) was employed for cDNA cloning of the CIP genes with some modifications. First-strand cDNAs were synthesized from ZYMV RNAs with minus strand oligonucleotide primers ZYMVC12M (5'-GGGGCGGCCGCTTTCTTGCTTTCAACGCGC-3') and ZYMVC14M (5'-GGGGCGGCCGCGCAAGTATGTTATTACCATGCTG-3') using murine leukemia virus (Moloney virus, MLV) reverse transcriptase (Gibco BRL). Double-stranded (ds) cDNAs were amplified from the first-strand ZYMV cDNAs using pairs of oligonucleotide primers ZYMVC12P (5'-GGGGCGGCCGCGCATGGTAATAACATACTTG-3') and ZYMVC12M (5'-GGGGCGGCCGCTTTCTTGCTTTCAACGCGC-3') or ZYMVC14P

(5'-GGGGCGGCCGCGCAAATAAAGCTGATGAAAATG-3') and ZYMVC14M (5'-GGGGCGGCCGCGCAAGTATGTTATTACCATGCTG-3') by the PCR method (Saiki *et al.*, 1988). The oligonucleotide primers were designed on the basis of nucleotide sequence of ZYMV-Cal RNA (Balint *et al.*, 1994). The amplified ds cDNAs were digested with *NotI* endonuclease and inserted into the *NotI* restriction site of Bluescript II SK<sup>+</sup> plasmid (Stratagene). The recombinant plasmids were introduced into *Escherichia coli* XL1-Blue and then extracted by the boiling method (Holms and Quigley, 1981). The DNAs were sequenced using ABI PRISM<sup>TM</sup> Dye Terminator Cycle Sequencing Ready Reaction Kit. Primers based on the nucleotide sequences of Bluescript II SK<sup>+</sup> plasmid [RV (5'-AACAGCTATGACCATG-3') and KS (5'-CGAGGTCGACGGTATCG-3')] and the ZYMV isolates [ZYMVC13P (5'-AAGCCTGGAAGTGCATTGC-3'), ZYMVC13M (5'-TGCAATGTTGTGCGCTGTGT-3'), and ZYMVC15P (5'-GCCATTTTCAAGATTTTGGACAA-3')] were used for sequencing. The nucleotide and deduced amino acid sequence analyses, similarity searches, and multiple alignments were carried out using the DNASIS program (Hitachi Software Engineering Co. Ltd. 1992). Phy-

		10	20	30	40	50	60	70	80	90	100
169		GLEDIESLED	DKRLTIDFDI	NTNDAQSSTT	FDVHFDDWN	RQLQQNRIVP	HYRTTGKFL	PTRTAAPVA	NEIASSEGE	FLVHGAVGSG	KSTSLPAHLA
M		-----	-----	-----E-----	-----	-----	-----	-----	-----	-----	-----
Cal		-----	-----	-----E-H-----	-----	-----	-----	-----	-----	-----	-----
S		-----N-----	-----	-----E-----	-----E-----	-----	-----	-----S-Y-----	-----	-----	-----
RI		-----N-----	-----K-----	-----E-----	-----	-----	-----	-----	-----N-----	-----	-----
		110	120	130	140	150	160	170	180	190	200
169		KKGKVLLEF	TRPLAENVSR	QLAGDFFQ	N VTLRMGLSC	FGSSNITVMT	SGFAPHYIVN	NPHQLMEPDF	VIIDECHVTD	SATIAFNAL	KEYNFAGKLI
M		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Cal		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
S		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----S-----
RI		-----	-----	-----	-----	-----Y-----	-----M-----	-----I-----	-----	-----	-----
		210	220	230	240	250	260	270	280	290	300
169		KVSATPPGRE	CDPDTQFAVK	VKTEDHLSFQ	AFVGAQKTGS	NADMVQHGN	ILVYVASINE	VDMLSKLLTE	RQFSVTRVDG	RTMQLGKTTI	ETHGTSQKPH
M		-----	-----	-----H-----	-----	-----	-----	-----	-----	-----	-----
Cal		-----	-----	-----H-----	-----	-----	-----	-----	-----	-----	-----
S		-----	-----	-----N-----	-----	-----	-----	-----	-----	-----	-----
RI		-----	-----	-----R-----	-----	-----I-----	-----	-----	-----	-----	-----
		310	320	330	340	350	360	370	380	390	400
169		FIVATNIEN	GVTLDVECVV	DFGLKVVAEL	DSENRVRYN	KKPVSYGERI	QRLGRVGRSK	PGTALRIGYT	EKGIESISEF	IATEAALSF	AYGLPVTTHG
M		-----	-----	-----	-----	-----S-----	-----	-----H-----	-----T-P-----	-----	-----
Cal		-----	-----	-----GRRT	QQR-----	-----S-----	-----	-----H-----	-----T-P-----	-----	-----
S		-----	-----	-----K-----	-----S-----	-----	-----	-----H-----	-----N-P-----	-----	-----S-----
RI		-----D-----	-----	-----S-----	-----N-----	-----	-----	-----H-----	-----N-P-----	-----	-----S-----
		410	420	430	440	450	460	470	480	490	500
169		VSTNIGKCT	VQMKRCALNF	ELTPFFTHL	IRHDGSMHPL	IHEELKQFKL	RDSEMLNKKV	ALPHQFVSQW	MDQSEYERIG	VHVQCHESTR	IPFTNGIPD
M		-----	-----	-----	-----	-----	-----	-----	-----G-----	-----	-----
Cal		-----	-----K-----	-----	-----	-----	-----	-----	-----	-----NS-----	-----
S		-----N-----	-----K-----	-----	-----	-----	-----	-----	-----	-----I-----	-----
RI		-----K-----	-----	-----	-----	-----	-----	-----	-----T-GD-H-----	-----I-N-N-----	-----
		510	520	530	540	550	560	570	580	590	600
169		KVYEIKMKCI	QENKNDALFG	KLSSACSTKV	SYTLSTDPAA	LPRTIAIIDH	LLAEEMMKRN	HPDTISSAVT	GYSFSLAGIA	DSFPRKRMRD	HTAHNAILQ
M		-----R-----	-----V-----	-----	-----	-----	-----	-----	-----	-----	-----Y-----
Cal		-----R-----	-----V-----	-----	-----	-----	-----	-----	-----	-----	-----Y-----
S		-----R-----	-----L-----	-----FPS-----	-----	-----	-----	-----M-----	-----	-----	-----H-----
RI		R-----	-----L-----	-----R-----	-----	-----	-----	-----M-----	-----	-----	-----
		610	620	630	634						
169		QARAQLLEPN	SKNVNINLS	DLEGIGVIKS	VVLQ						
M		-----	-----	-----	-----						
Cal		-----	-----	-----	-----						
S		-----	-----	-----	-----						
RI		-----D-----	-----	-----	-----						

Fig. 2

## Multiple alignment of deduced amino acid sequences of CIP genes of ZYMV isolates

Dashes show identical amino acids in comparison to ZYMV-169. The site of the so-called nucleotide binding motif is boxed (aa 85–93). The sources of CIP gene sequence data are Balint *et al.*, 1994 (ZYMV-Cal), Baker *et al.*, 1994 (ZYMV-RI), and Lec *et al.*, 1997 (ZYMV-S).

logenic analysis of the deduced amino acid sequences of CIP genes was accomplished using the Protein Sequence Parsimony Method (PROTOPARS) of Phylogeny Inference Package (PHYLIP) as developed by Felsenstein (1993). For phylogenetic analysis, an ordinary strain (PVY-O) of potato virus Y (Ohshima *et al.*, 1993) was defined as outgroup. All data sets were subjected to bootstrap analysis by performing 100 replications using SEQBOOT in PHYLIP. The tree depicted was unrooted and both the horizontal and vertical branch lengths were arbitrary.

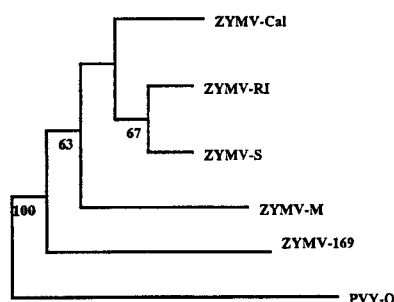
The nucleotide sequences of the ZYMV-169 and ZYMV-M CIP genes were determined from nine recombinant clones and will appear in the DDBJ, EMBL and GenBank nucleotide sequence data bases under Acc. Nos. AB020477 and AB020478, respectively. No differences were observed in overlapping regions of these clones. Nucleotide sequences of the cDNAs including a portion of P3 gene, 6K1 gene, the complete CIP gene, and a portion of 6K2 gene with deduced amino acid sequences are shown in Fig. 1. The protease cleavage site between the 6K1 and CIP genes was Q/G, and that between the CI and 6K2 genes was Q/S (Fig. 1). The CIP genes of ZYMV-169 and ZYMV-M were 1902 nu-

cleotides in length, and encoded 634 amino acids (Fig. 1). The size of the CIP genes of ZYMV-169 and ZYMV-M was identical to that of other ZYMV isolates reported previously. Comparison of the nucleotide and deduced amino acid sequences of the CIP genes of ZYMV-169 and ZYMV-M revealed 91% and 98% similarities, respectively. A total of 163 nucleotide substitutions were present between the CIP genes of the two isolates and gave rise to 10 amino acid differences (Fig. 1). The nucleotide substitutions were present predominantly at the third base of the codon. A nucleotide binding motif with consensus sequence G/AXXXG KS/T (Lain *et al.*, 1989) was fully conserved in ZYMV-169 and ZYMV-M CIPs at aa 85–93 (Fig. 2). The nucleotide and deduced amino acid sequences of the CIP genes of Japanese ZYMV isolates were compared with those of previously reported ZYMV isolates. ZYMV-169 showed 82–91% nucleotide similarity and 95–96% amino acid similarity to other ZYMV isolates (Table 1) and had unique amino acids at positions 24, 369, 376, 378, and 518 (Fig. 2). On the other hand, ZYMV-M showed 81–97% nucleotide similarity and 95–97% amino acid similarity to other

**Table 1. Percentage similarity of the nucleotide and deduced amino acid sequences of potyvirus CIP genes**

	ZYMV-169	ZYMV-M	ZYMV-Cal	ZYMV-RI	ZYMV-S	PVY-O	PRSV-H	TuMV-J
ZYMV-169		91	90	82	86	61	61	64
ZYMV-M	98		97	81	85	62	61	63
ZYMV-Cal	96	97		82	85	62	61	63
ZYMV-RI	95	95	93		82	61	62	63
ZYMV-S	96	96	95	95		61	61	63
PVY-O	52	53	53	51	52		62	62
PRSV-H	55	55	54	54	55	54		62
TuMV-J	55	55	54	54	54	56	55	

The upper diagonal of this table shows the nucleotide similarities while the lower diagonal shows the amino acid similarities. The sources of the sequence data were: Balint *et al.*, 1994 (ZYMV-Cal), Baker *et al.*, 1994 (ZYMV-RI), Lee *et al.*, 1997 (ZYMV-S), Ohshima *et al.*, 1993 (PVY-O), Yeh *et al.*, 1992 (PRSV-H) and Ohshima *et al.*, 1996 (TuMV-J).

**Fig. 3**

**Phylogenetic relationships among five ZYMV isolates based on multiple alignment of the deduced amino acid sequences of CIP genes**  
The ZYMV isolates are those shown in Fig. 2. The tree was obtained by the Protein Sequence Parsimony Method of PHYLIP with PVY-O (Ohshima *et al.*, 1993) defined as the outgroup. The tree shown is unrooted and both the vertical and horizontal branch lengths are arbitrary. Values at the nodes indicate the percentages of bootstrap analyses supporting the grouping. Bootstrap percentages below 50% are not shown.

ZYMV isolates (Table 1) and had unique amino acid at position 472 (Fig. 2). When the CIP genes of ZYMV and other potyviruses were compared, the nucleotide and amino acid sequence similarities ranged between 61–64%, and 51–56%, respectively (Table 1). These values are similar to those of other members of the *Potyvirus* genus (Shukla and Ward, 1988). A phylogenetic analysis derived from the deduced amino acid sequences of ZYMV CIP genes supported by low bootstrap values (less than 90%) showed that all the five ZYMV isolates clustered into one group (Fig. 3).

The above results indicate that the CIP genes of Japanese ZYMV isolates are very close to those of other ZYMV isolates (Figs. 2 and 3), although ZYMV-169 was shown to be the most distinct isolate compared to other ZYMV isolates when amino acid sequences of their CPs were analyzed by phylogenetic tree (Kundu *et al.*, 1997). Sequence similarities in different regions of the genomes of distinct potyvi-

rus and strains showed that CIP is the most conserved protein after the RNA-dependent RNA polymerase (NIb gene) (Ward *et al.*, 1992).

**Acknowledgements.** We thank Dr. M. Kusaba (Laboratory of Plant Pathology, Saga University) for his help in phylogenetic analysis. We also thank the Saga University for the provision of the Structure-Function Analysis System for Useful Biological Macromolecules.

## References

- Baker CA, Marlow GC, Wisler GW, Lecoq H, Hiebert E (1994): Comparative sequence analysis of zucchini yellow mosaic potyvirus (ZYMV) from Reunion Island with other ZYMV isolates. *GenBank Accession No. L31350*.
- Balint R, Plooy I, Steele C (1994): The nucleotide sequence of zucchini yellow mosaic potyvirus. *GenBank Accession No. L29569*.
- Dougherty WG, Carrington JC (1988): Expression and function of potyviral gene products. *Annu. Rev. Phytopathol.* **26**, 123–143.
- Dougherty WG, Semler BL (1993): Expression of virus-encoded proteinases: functional and structural similarities with cellular enzymes. *Microbiol. Rev.* **57**, 781–822.
- Edwardson JR (1974): Some properties of the potato virus Y group. *Fla. Agric. Exp. Stn. Monogr. Ser.* **4**.
- Felsenstein J (1993): PHYLIP (*Phylogeny Inference Package*), Version 3.5c. University of Washington, Seattle.
- Hari V, Seigel A, Rozek A, Timberlake WE (1979): The RNA of Tobacco etch virus contains poly(A). *Virology* **92**, 568–571.
- Hari V (1981): The RNA of Tobacco etch virus: further characterization and detection of protein linked to RNA. *Virology* **112**, 391–399.
- Holms DS, Quigley M (1981): A rapid boiling method for the preparation of bacterial plasmids. *Anal. Biochem.* **114**, 513–524.
- Kundu AK, Ohshima K, Sako N (1997): Nucleotide sequences of the coat protein genes of two Japanese zucchini yellow mosaic virus isolates. *Acta Virol.* **41**, 297–301.

- Lain S, Riechmann JL, Martin MT, Garcia JA (1989): Homologous potyvirus and flavivirus proteins belonging to a superfamily of helicase-like proteins. *Gene* **82**, 357–362.
- Lain S, Martin MT, Riechmann JL, Garcia JA (1991): Novel catalytic activity associated with positive-strand RNA virus infection: nucleic acid stimulated ATPase activity of the plum pox potyvirus helicase protein. *J. Virol.* **65**, 1–6.
- Lee KC, Parvesh HM, Chng CG, Wong SM (1997): Sequence and phylogenetic analysis of the cytoplasmic inclusion protein gene of zucchini yellow mosaic potyvirus: its role in classification of the *Potyviridae*. *Virus Genus* **14**, 41–53.
- Lindbo JA, Dougherty WG (1994): In Webster RG, Granoff A (Eds): *Encyclopedia of Virology*. Vol. 3. Academic Press, San Diego, pp. 1148–1153.
- Lisa V, Lecoq H (1984): Zucchini yellow mosaic virus. *CMI/AAB Descriptions of Plant Viruses*, No. **282**.
- Ohshima K, Inoue AK, Shikata E (1993): Molecular cloning, sequencing, and expression in *Escherichia coli* of potato virus Y cytoplasmic inclusion gene. *Arch. Virol.* **128**, 15–28.
- Ohshima K, Matsuo K, Sako N (1994): Nucleotide sequences and expression in *Escherichia coli* of the coat protein genes from two strains of melon necrotic spot. *Arch. Virol.* **138**, 149–160.
- Ohshima K, Tanaka M, Sako N (1996): The complete nucleotide sequence of turnip mosaic virus RNA Japanese strain. *Arch. Virol.* **141**, 1991–1997.
- Rodríguez-Cerezo E, Shaw JG (1991): Two newly detected non-structural proteins in potyvirus-infected cells. *Virology* **185**, 572–579.
- Rosner A, Gingburg I, Bar-Joseph M (1983): Molecular cloning of complementary DNA sequences of citrus tristeza virus RNA. *J. Gen. Virol.* **71**, 1451–1460.
- Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988): Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **230**, 1350–1354.
- Sako N, Matsuo K, Nonaka F (1980): Purification of watermelon mosaic virus. *Ann. Phytopathol. Soc. Jpn.* **46**, 639–646 (in Japanese).
- Shukla DD, Ward CW (1988): Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. *J. Gen. Virol.* **69**, 2703–2710.
- Shukla DD, Ford RE, Tosic M, Jilka J, Ward CW (1989): Possible members of the potyvirus group transmitted by mites or white flies share epitopes with aphid-transmitted definitive members of the group. *Arch. Virol.* **105**, 143–151.
- Ward CW, McKern NM, Frenkel MJ, Shukla DD (1992): Potyvirus taxonomy. In Barnett OW (Ed.): *Arch. Virol.* (Suppl. 5). Springer, Wein - New York, pp. 282–297.
- Yeh SD, Gonsalves D (1984): Purification and immunological analyses of cylindrical inclusion protein induced by papaya ringspot virus and water melon mosaic virus I. *Phytopathology* **74**, 1273–1278.
- Yeh SD, Jan FJ, Chiang CH, Doong TJ, Chen MC, Chung PH, Bau HJ (1992): Complete nucleotide sequence and genetic organization of papaya ringspot virus RNA. *J. Gen. Virol.* **73**, 2531–2541.