Nucleotide Sequences of Coat Protein and 17 K Protein Genes for a Potato Leafroll Virus Japanese Isolate*

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Key words: potato leafroll virus, coat protein, 17 K protein, nucleotide sequence.

Luteovirus, is a large group of plant viruses, which cause economically important diseases in a wide range of host⁹⁾, including barley yellow dwarf virus (BYDV), beet western yellows virus (BWYV) and potato leafroll virus (PLRV)^{2,15)}. They are transmitted in a persistent manner by aphids to host plants where the viruses replicate specifically in the phloem tissues. PLRV is an isometric particle with a diameter of 25-nm. The genome consists of a single-stranded plus-sense RNA with a genome-linked viral protein (VPg). No poly A tail is reported to follow at 3' end of the RNA. A sequence of 5987 was first reported for the genome of PLRV Scotland isolate by Mayo *et al.*¹⁰⁾ The sequence contained six large open reading frames including a 23 K coat protein (nucleotides 3693 to 4316; 208 amino acids) and a 17 K polypeptide (nucleotides 3718 to 4185; 156 amino acids) which was encoded in a different frame¹⁰⁾. It has been proposed by Miller *et al.*¹³⁾ that 17 K protein may code for the VPg, based on its similarity in size to that of the VPg isolated from BYDV¹⁴⁾. As the VPg of PLRV has been reported to be about 7 K¹¹⁾, it has been suggested by van der Wilk *et al.*²¹⁾ that 17 K protein might be a precursor polypeptide which is proteolytically processed to yield a 7 K protein. In 1991, Tacke *et al.*¹⁸⁾ reported that this protein bound a single-stranded nucleic acid. Recently, nucleotide sequences for the isolates of PLRV coat proteins from the Netherlands²¹⁾, Federal Republic of Germany¹⁹⁾, Canada and Australia⁶⁾ have been established. In this report, we describe the nucleotide sequence of coat protein and 17 K protein genes for a Japanese isolate (PLRV-J) of PLRV and compare those predicted amino acid sequences to those of PLRV isolates, BYDV and BWYV previously reported.

PLRV-J isolated from a potato plant in Hokkaido, Japan, was propagated on *Physalis floridana* in a greenhouse of Hokkaido University and purified by the method described by Takanami and Kubo²⁰⁾. The genomic RNA was extracted by the procedure described by Veidt *et al.*²²⁾ The first-strand cDNAs were synthesized from PLRV-J RNA according to the method of Gubler and Hoffman⁴⁾ using the minus strand oligonucleotide primers PLCP14M (5'-GCTGCAGAGCTCTATTTGGGGTTTTCGAAAGC-3') or PLCP25M (5'-GGCTTGCATGCTCGATTGA-3'). Double (ds) stranded cDNAs were amplified from the first-strand cDNA using two pairs of oligonucleotide primers: PLCP12P (5'-GAACACTGAAGGAGC-TACTA-3') and PLCP14M, or PLCP18P (5'-GAAGAGGAGGCAATCGC-3') and PLCP25M by polymerase chain reaction (PCR) method as described by Saiki *et al.*¹⁶⁾ PCR was performed using Taq DNA polymerase (Takara Shuzo Co., Ltd.) and the amplification proceeded through a cycle of denaturation at 94°C (1 min), annealing at 50°C (2 min) and primer extension at 72°C (3 min) for a total of 26 cycles by using Program Temp Control System PC-700 (Astec Inc.). The amplified cDNAs were digested with restriction endonucleases *Sac*I, or *Bam*HI and *Sph*I and ligated into *Sac*I or *Bam*HI and *Sph*I restriction sites of plasmid pUC119. The recombinant plasmids were introduced into *E. coli* JM109. The nucleotide

* This work was supported in part by grants from The Akiyama Foundation and from The Suhara Memorial Foundation.

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sequence was determined from the PCR-mediated clones. The DNAs were sequenced by dideoxynucleotide method¹⁷⁾ using alkaline-denatured plasmid DNAs. The sequence primers used in this experiment were M13 Primer M4 (Takara Shuzo Co., Ltd.), M13 Primer RV (Takara Shuzo Co., Ltd.),

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10      20      30      40      50      60      70      80      90
AATAGCCAAGCACACGAGTTGCAAGCATTGGAAGTTTAAGCCTCGTCACACCAACCGGACAAAATAGATTATAAATTCTTAGCGGGAT

100     110     120     130     140     150     160     170     180
TTGCTTTAGGATTCTCATCCGCAATCCCATTTCAGTAGCCGGTTTATATTTAGTTTACCTAAAGATTTCCTCCCACGTGCGATCAATTG

190     200     210     220     230     240     250     260     270
TTAATGAGTACGGTCGTGGTTAAAGGAAATGTCAATGGTGGTGTAACAACAAGGCGAAGAAGGCAATCCCTTCGCAGGCGCGCT
M S T V V V K G N V N G G V Q Q P R R R R R R Q S L R R R A
M S M V V Y N N Q E G E E G N P F A G A L

280     290     300     310     320     330     340     350     360
AACAGAGTTCAGCCAGTGGTTATGGTCACGGCCCTGGGCAACCCAGGCGCGGAAGACGTAGAAGAGGAGGCAATCGCCGCTCAAGAAGA
N R V Q P V V M V T A P G Q P R R R R R R R R G G N R R S R R
T E F S Q W L W S R P L G N P G A E D V E E E A I A A Q E E

370     380     390     400     410     420     430     440     450
ACTGGAGTTCGCCGAGGACGAGGCTCAAGCGAGACATTCGTGTTTACAAAGGACAACCTCGTGGGCAACTCCCAAGGAAGTTTCACTTC
T G V P R G R G S S E T F V F T K D N L V G N S Q G S F T F
L E F P E D E A Q A R H S C L Q R T T S W A T P K E V S P S

460     470     480     490     500     510     520     530     540
GGGCCGAGTCTATCAGACTGTCCGGCATTCAAGGATGGAATACTCAAGGCCTACCATGAGTATAAGATCACAAGCATCTTACTTCAGTTC
G P S L S D C P A F K D G I L K A Y H E Y K I T S I L L Q F
G R V Y Q T V R H S R M E Y S R P T H S I R S Q A S Y F S S

550     560     570     580     590     600     610     620     630
GTCAGCGAGGCCTCTTCCACCTCCTCCGGCTCCATCGCTTATGAGTTGGACCCCATTTGCAAAGTATCATCCCTCCAGTCTACGTCAAC
V S E A S S T S S G S I A Y E L D P H C K V S S L Q S Y V N
S A R P L P P P P A P S L M S W T P I A K Y H P S S P T S T

640     650     660     670     680     690     700     710     720
AAGTTCCAAATTACGAAGGGCGGCGCAAACTTATCAAGCGCGGATGATAAACGGGGTAGAATGGCACGATTCTTCTGAGGATCAGTGC
K F Q I T K I G A K T Y Q A R M I N G V E W H D S S E D Q C
S S K L R R A A P K L I K R G *

730     740     750     760     770     780     790     800     810
CGGATACTGTGGAAGGGAATGGAAAATCTTCAGACACCGCAGGATCCTTCAGAGTCACCATCAGGGTGGCTTTGCAAAAACCCCAATAG
R I L W K G N G K S S D T A G S F R V T I R V A L Q N P K *

820     830     840     850     860     870     880     890     900
GTAGACTCCGGATCAGAGCCTAGTCCAAGCCCAACCAACCCACTCCAACCTCCCAAGACGAGCGATTATTGCTTACGTTGGC

910     920     930     940     950     960     970     980     990
ATACCTATGCTAACCATTCAGGCGAGGGAACGACGACCAAAATCATATTGGGTTCTTAGGGAGCCAAAGGATGAAATATATAGAGGAC

1000    1010    1020    1030
GAGAACCAAGATTATACAAAGTTTGTCTGAGTATTACTC

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Fig. 1. Nucleotide and predicted amino acid sequences of coat protein and 17 K protein genes of PLRV-J. Upper lane shows nucleotide sequence of the coat protein and 17 K protein genes. Middle and lower lanes show predicted amino acid sequences of the coat protein and 17 K protein, respectively.

PLCP17M (5'-GGGGGTCCAACCTCATTA-3'), PLCP18P and PLCP22P (5'-GTGGTGTACAACAACCA-AG-3'). All the primers used in this experiment corresponded to the nucleotide residues from 5' end as described by Mayo *et al.*¹⁰⁾ (PLCP12P: 3487 to 3507, PLCP14M: 4299 to 4319, PLCP17M: 4088 to 4104, PLCP18P: 3841 to 3857, PLCP22P: 3727 to 3745 and PLCP25M: 4551 to 4559). The PLRV-J sequence was determined from several overlapping clones (pUCPLCPSS1, 2 and 3 or pUCPLCPBS1 and 2). All of the sequence was determined at least twice from these clones in both orientations and no differences was observed in overlapping regions of these clones. Computer analyses were carried out using SDC-GENETYX computer programs (Software Development, Co. Ltd.).

The complete nucleotide and predicted amino acid sequences of PLRV-J coat protein and 17 K protein are aligned in Fig. 1. We concluded these two proteins to be coat protein and 17 K protein because high nucleotide and amino acid sequence homologies were obtained between PLRV-J and PLRV isolates previously reported^{6,10,19,21)} (Tables 1 and 2). Numbers of the nucleotides encoding coat protein and 17 K protein genes were 624 and 465, and numbers of the predicted amino acids were 208 and 155, respectively.

Comparison of percent homology in the predicted amino acid sequences among PLRV-J coat protein and those of PLRV isolates, and those of luteoviruses previously reported are shown in Table 1. More than 96% homology of the amino acid sequences of coat proteins were observed among PLRV isolates, indicating that PLRV isolates so far reported in different countries had highly conserved coat protein sequences. On the other hand, the predicted amino acid sequences of coat protein genes of BWYV and BYDV, showed 63-65% and 46-49% sequence homology to that of PLRV, respectively. The results provided an added support for the division of luteoviruses into two subgroups, as proposed by Veidt *et al.*²²⁾ and Martin *et al.*⁸⁾ The results also corresponded to the serological reactivities by polyclonal and monoclonal antibodies that PLRV is more closely related to BWYV than to BYDV (unpublished data).

As shown in Table 2, more than 93% homology in amino acid sequence of 17 K protein of the PLRV

Table 1. Percent homology in amino acid sequences of coat proteins from PLRV isolates and luteoviruses

	J	S	C	A	N	D	BWYV	BYDV
PLRV-J	—	98.1	99.5	98.1	98.1	99.0	64.9	49.0
PLRV-S		—	98.1	96.6	96.2	97.1	64.7	47.5
PLRV-C			—	98.6	97.6	98.6	64.9	48.5
PLRV-A				—	96.2	97.1	64.4	49.0
PLRV-N					—	98.1	63.4	48.5
PLRV-D						—	64.9	47.7
BWYV							—	46.2
BYDV								—

a) The sources of sequences data are: PLRV-S¹⁰⁾, PLRV-A and PLRV-C⁹⁾ PLRV-N²¹⁾, PLRV-D¹⁹⁾, BWYV²²⁾ and BYDV¹²⁾.

Table 2. Percent homology in amino acid sequences of 17 K proteins from PLRV isolates and luteoviruses

	J	S	C	A	N	D	BWYV	BYDV
PLRV-J	—	98.1	98.7	96.1	96.8	99.4	52.6	33.8
PLRV-S		—	99.4	94.2	97.4	97.4	56.3	33.8
PLRV-C			—	94.8	98.1	98.1	53.3	33.8
PLRV-A				—	92.9	95.5	53.3	33.6
PLRV-N					—	96.1	53.3	33.8
PLRV-D						—	55.6	33.8
BWYV							—	30.1
BYDV								—

a) The sources of sequence data are: PLRV-S¹⁰⁾, PLRV-A and PLRV-C⁹⁾ PLRV-N²¹⁾, PLRV-D¹⁹⁾, BWYV²²⁾ and BYDV¹²⁾.

isolates were observed. On the other hand, percent homology in the predicted amino acid sequences of 17 K protein between PLRV-J and BWYV showed higher homology than that between PLRV-J and BYDV. The result also indicated that PLRV is more closely related to BWYV than to BYDV.

Recent studies on transgenic plants expressing the coat protein genes of several plant viruses resulted in reduced virus synthesis following specific exposure to viruses^{1,3,5,7)}. Therefore, to produce PLRV resistant plants, further studies on transgenic plants of PLRV-J coat protein gene are now under way.

We thank Dr. I. Uyeda, Department of Agro-biology and Bio-resources, Faculty of Agriculture, Hokkaido University for critical reading of manuscript.

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和 文 摘 要

大島一里・中屋隆明・松村 健・四方英四郎・木村郁夫：本邦で分離したジャガイモ葉巻ウイルス外被タンパク質および17 K タンパク質遺伝子の塩基配列

本邦で分離したジャガイモ葉巻ウイルス(PLRV-J)の外被タンパク質および17 K タンパク質遺伝子の塩基配列を決定した結果、塩基数はそれぞれ624 および465 であり、アミノ酸数は、208 および155 と推定された。PLRV-Jと諸外国で分離された PLRV 分離株間の外被タンパク質および17 K タンパク質のアミノ酸配列の相同性を比較すると、両タンパク質ともに90%以上の高い相同性を示した。同様に PLRV-J とテンサイ西部萎黄ウイルス(BWYV)およびオオムギ黄萎ウイルス(BYDV)と比較すると BYDV(外被タンパク質：49.0%, 17 K タンパク質：33.8%)に比べて BWYV(外被タンパク質：64.9%, 17 K タンパク質：52.6%)との間でより高い相同性を示した。

(Received September 7, 1992)