

Passionfruit woodiness virus-奄美大島株(PWV-AO)の外被タンパク質遺伝子ならびに3'末端非翻訳領域の塩基配列

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Nucleotide Sequence of the Coat Protein Gene and 3'-noncoding Region of the Passionfruit Woodiness Virus-Amami Ohshima Isolate*

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Key words : passionfruit woodiness virus, coat protein gene, 3'-noncoding region.

A potyvirus isolated from the passionfruit hybrid cultivar Ohdama (*Passiflora edulis* × *P. edulis* f. *flavicarpa*) collected from the Amami main island of Kagoshima Prefecture has been previously described as the passionfruit woodiness virus-Amami Ohshima isolate (PWV-AO)¹⁰⁾.

In this report, we describe the cDNA cloning and nucleotide sequencing of the 3'-terminal region of PWV-AO RNA.

We purified virus particles from infected Ohdama leaves (200 g) as previously described¹⁰⁾. The RNA was extracted from the purified viruses using proteinase K and SDS followed by a phenol-chloroform extraction and ethanol precipitation¹³⁾. Two μ g of RNA was used for the cDNA synthesis. cDNA was synthesized by the method of Gubler and Hoffman⁶⁾ using a cDNA Synthesis System Plus kit (Amersham) and the ligated *EcoRV* site of pBluescript II SK+ (Stratagene). Competent *Escherichia coli* JM109 cells were transformed with the hybrid plasmids according to the method of Hanahan⁷⁾. Plasmid preparations were made by the alkaline-SDS lysis method¹⁾. Plasmids and restriction digests were analyzed by electrophoresis in 1% agarose and by southern blot hybridization with a viral RNA probe using the ECL detection system (Amersham). One oligo dT-primed cDNA clone pPW6 with an insert size of 1.6 kbp was selected. The nucleotide sequences of subclones containing progressive unidirectional deletions⁸⁾ of pPW6 fragments, which were obtained using the Erase-A-base system (Promega), were determined by the dideoxynucleotide chain-termination method¹⁶⁾ using M13 and T7 primers with a DNA sequencer model 373A (ABI). Computer analyses of the nucleotide and amino acid sequences were performed using the PC floppy version program from SDC-GENETYX (SDC software).

The C terminal portion of the PWV-AO polyprotein and the complete 3' non-coding region (NCR) are shown in Fig. 1. The dipeptide QT located at amino acid position 153 has been selected as a putative cleavage site for the coat protein (CP) of PWV-AO with the help of

the conserved upstream residue motif (CCESVSLQ)^{3,11)}. Cleavage at the selected dipeptide generates a predicted CP which is 290 residues long (32.7 kDa in size), larger than the respective values of 269 (30.1 kDa) for PWV-TB (Tip Blight strain)¹⁸⁾ and 278 (31.0 kDa) for PWV-K (Severe strain)⁵⁾, and smaller than the CP for PWV-AO (35 kDa) determined by SDS-PAGE¹⁰⁾. The latter discrepancy is to be expected because the relative molecular weight of a plant virus CP, estimated by SDS-PAGE, is inclined to differ from the weight determined by the amino acids analyses by as much as 10%¹⁷⁾. The deduced amino acid sequence of the PWV-AO CP contained Asp-Ala-Asn (DAG) tripeptides at positions 169 to 171 and 211 to 213, which may correlate with the high frequency of aphid transmissibility⁹⁾. The open reading frame of PWV-AO was followed by a 258 nucleotide NCR [excluding the poly(A) tail]. NCR contained the consensus AG-GTGG-----CCACC sequence of "the second group" suggested by Uyeda¹⁹⁾, at positions 1416 to 1432. Table 1 compares the CP amino acid and 3'-NCR sequences of PWV-AO and each of the PWV strains^{5,18)}, as well as 6 different potyviruses^{2-4,11,14,15)} of "the second group"¹⁹⁾. Percent homologies shown in Table 1 and discussed in the following paragraph were computed using HOMOGAPP and HOMOGAPN programs, except for 'the standard percent for identification' which is cited from Shukla and Ward. At present the CP sequences for six potyviruses which cause 'woodiness' symptoms, including PWV-AO, have been proposed. PWV-M (Mild strain), -S (another Severe strain) and -TB have a high sequence identity reciprocally (95-99%), regardless of the different appearance of their symptoms, and can be united as strains of the same PWV, or as a "PWV-TB units". PWV-K shares 82-84% CP amino acid sequence identities with the PWV-TB units. However, PWV-AO shares only 75.6, 74.8 and 76.5% CP amino acid sequence identities with PWV-K⁵⁾, PWV-M and PWV-S/-TB¹⁸⁾, respectively. These values are not significantly different from the soybean mosaic virus (SMV)-G2¹¹⁾ (75.2%) or the strains of watermelon

* The nucleotide sequence data reported here will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases under the accession number D85849.

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1	--CTATGACACACTCAGTGCTTCTTTGCTGAAGTGGACTGAACATAATTCGATGATCGATCAAAACGGCGAGAAGATTGTGGTTT	90
1	X Y D T L S A S F A E L G L N Y N F D D R S K R R E D L W F	30
91	ATGTCTCATACAGCAATTGAAATTGATGGAGTGTACATCCCAAGCTCGAACCTGAACGAATTGTTTCAATACTAGAATGGGATAGGAGT	180
31	M S H T A I E I D G V Y I P K L E P E R I V S I L E W D R S	60
181	AAAGAAATTATGCACAGGACTGAGGCAATTTGCGCACGAATGATTGAAGCATGGGGTTATACAGATTGCTGCGTGAAATCAGAAAGTTT	270
61	K E I M H R T E A I C A R M I E A W G Y T D L L R E I R K F	90
271	TACCTGTGGCTTGTTCAAAAGACGAGTTCAAGGAAGTGTGCGAGCTGGCAAAGCACCATACATTGCAGAAACAGCTTTGAGAAAGTTG	360
91	Y L W L V Q K D E F K E L A A A G K A P Y I A E T A L R K L	120
361	TACATGAATAAGGATGCTAGCATGGATGAGCTACAGAAATACTGCGATGCTAGACCTCGAGCATGTGGAAGGTTGCTGTGAATCAGTA	450
121	Y M N K D A S M D E L Q K Y L H V L D L E H V E G C C E S V	150
451	TCTTTGCAACCAATCAGAAGAAAACAAAGACGAAGTGGAAAGGATGCAATTGATGCAGGCAATGATGGGAAAAGGAAAGACAAGGAA	540
151	S L Q T K S E E N K D E L G K D A I <u>D A G</u> N D G K R K D K E	180
541	AAGGAAGGAATGGTCACACCCCGACGAATCCAAATCCCAATAATTCAAATTTGGTGGTAGCTCATTTAACAGGAAAGACAGGATGTC	630
181	K E G M V T P P T N P N P N N S K F G G S S F N R K D R D V	210
631	GATGCTGGCTCAAAGGTAAATGGTACCACGGCTGCAAAGATCACAAGAAGATGAATCTACCAACTGTGAAAGGTAGGGCCATACTC	720
211	<u>D A G</u> S K G K M V P R L Q K I T K K M N L P T V K G R A I L	240
721	GACTTGGATCATTTACTTGAGTATGCACCAATCAAGTTGATCTCTTTAATACCAGAGCTACAAAGTCACAATTTGAATCATGGTATTCA	810
241	D L D H L L E Y A P N Q V D L F N T R A T K S Q F E S W Y S	270
811	GCAGTCCAAAGAGAATATGAGCTGGATGACAATCAAATGAGCGTCATCATGAATGGTTTCATGGTGGTGCATTGACAATGGCACTTCA	900
271	A V Q R E Y E L D D N Q M S V I M N G F M V W C I D N G T S	300
901	CCAAATGTTAATGGCAGATGGGTGATGATGGACGGAGAAGGACAAATTGAATACCCATTGAAGCCGTTAGTTGAAAATGCCAGCCAACA	990
301	P N V N G T W V M M D G E G Q I E Y P L K P L V E N A Q P T	330
991	TTGAGACAAATATGCATCACTTTTCAGATGCGGCTGAGGCATACATTGAGATGAGAAATCCAAAGAGCCGTATATGCCTAGATATGGT	1080
331	L R Q I M H H F S D A A E A Y I E M R N S K E P Y M P R Y G	360
1081	ACTCTGAGGAATTTGAGGGACTTGAGTTTGGCGAGGTATGCTTTTGATTCTATGAAGTAACCTCCAAGACGCCTAACAGAGCAAGAGAA	1170
361	T L R N L R D L S L A R Y A F D F Y E V T S K T P N R A R E	390
1171	GCGGTAGCACAAATGAAAGCCGCTGCCCTCGCAACGTTTCTACTAGGTTGTTTGGCCTGGATGGAATGTTTCAACCACTGGCGAGAAC	1260
391	A V A Q M K A A A L A N V S T R L F G L D G N V S T T G E N	420
1261	ACTGAAAGGCATACTGCACGGGACGTCAATCAGAACATGCACACCCCTTTGGGTATGAATCCAGCAGTGAAGGTTGGGTAAACCGACC	1350
421	T E R H T A R D V N Q N M H T L L G M N S Q Q *	443
1351	ACAGTTGCTTTCTCGTTTTGCCTTTAAATTTCTATAGTAGTATAACTTTCACTCTCTTTAAGTTCAAGTGGTTACACCACCTTCCTGCA	1440
1441	AACCTATTGTGATAGAGTGGCTCAGCCACAGTGTGTTGGTAACTATAGTTTAAAGTAAGGGCGGGGAGAACCCTTGCAACGCCGGAGC	1530
1531	TTTCAGAGTGGATGCTTCCACGTGACTGTCCGAGTTTGGCAACGTTTGTGTCT(A) _n	1587

Fig. 1. Nucleotide and deduced amino acid sequences of the 3'-terminal regions of the PWV-Amami Ohshima isolate. An arrowhead indicates the cleavage site recognized by the proteinase. An asterisk indicates the position of the termination codon. The vector transmission recognition sequences in CP are underlined with a straight line. The consensus sequence of the second group¹⁹⁾ in NCR is underlined with a wavy line.

mosaic virus 2 (WMV2)^{3,15)} (73.6 and 72.9%). Furthermore, a comparison of the amino acid sequence, excluding the N-terminus (the variable region), of PWV-AO with that of the SMV and WMV2 strains revealed an 82.4 and 84.1% identity, which is much higher than that

of PWV-S/-TB (76.9%), PWV-K (75.8%) and PWV-M (75.6%). In contrast, PWV-AO shares 64 to 66% 3'-NCR sequence identities with the SMV-G2 and WMV2 strains, which are significantly lower than the 79 to 80% identity between the SMV-G2 and WMV2 strains.

Table 1. Percentage identities of the coat protein amino acid sequence and the 3' non-coding region nucleotide sequence for a comparison of the passionfruit woodiness virus-Amami Ohshima (PWV-AO) isolate and other potyviruses^{a)}

Virus	Coat protein		3' Noncoding region
	Whole amino acids	Amino acids excluding the N-terminus	Nucleotides
PWV-S	76.5	76.9	na ^{b)}
PWV-TB	76.5	76.9	na
PWV-K	75.6	75.8	na
SMV-G2	75.2	82.4	66.3
PWV-M	74.8	75.6	na
WMV2-Aus	73.6	84.1	63.6
WMV2-USA	72.9	84.1	64.4
SAPV	69.4	77.1	56.5
PRSV-W	58.1	65.0	50.3
JGMV	56.2	64.7	nof ^{c)}

a) Sequence data were analyzed using HOMOGAPP and HOMOGAPN programs of the SDC-GENETYX (SDC software) for coat proteins and nucleotides, respectively. The sources for the sequences are as follows: PWV-S, -TB and -M, Shukla and Ward (1989); PWV-K, Gough and Shukla (1992); SMV-G2, Jayaram *et al.* (1991); WMV2-Aus, Frenkel *et al.* (1989); WMV2-USA, Quemada *et al.* (1990); SAPV, Brand *et al.* (1993); PRSV-W, Quemada *et al.* (1990); JGMV, Gough *et al.* (1987).

b) na; Data of 3' untranslated regions for PWV-S, -TB, -K and -M are not available.

c) nof; No object found. Homology score less than 49%.

The South African passiflora virus²⁾ (SAPV, recently identified as a strain of the cowpea aphid-borne mosaic virus¹²⁾) has only a 69-71% sequence identity with the PWV-AO, -K and -TB units, yet it also causes the typical 'woodiness' symptoms. According to Shukla and Ward¹⁸⁾ CP amino acid sequence data can be used to identify and differentiate distinct potyviruses and their strains. In their analysis, the sequence identity between distinct members ranges from 38 to 71% (average 54%) while that between strains of one virus ranges from 90 to 99% (average 95%). From this point of view, the "PWV subgroup" is exceptional. The percent homologies for this group suggest that its members belong to neither of the aforementioned two categories. According to the present authoritative taxonomy^{18,20)}, the PWV-AO, -K and -TB units are too distantly related to be of the same strains, yet they cannot be differentiated as distinct viruses. Furthermore, from the same level of CP homologies, PWV, SMV and WMV2 can possibly be related taxonomically. A consensus sequence of NCR¹⁹⁾ may partially support this hypothesis.

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和 文 摘 要

岩井 久・酒井 淳一・花田 薫・荒井 啓: Passionfruit woodiness virus-奄美大島株 (PWV-AO) の外被タンパク質遺伝子ならびに 3'末端非翻訳領域の塩基配列

passionfruit woodiness virus-奄美大島株 (PWV-AO) のゲノム RNA の 3'末端を含む領域約 1.6 kb をクローニングし、外被タンパク質 (CP) 遺伝子および 3'末端の非翻訳領域 (NCR) の塩基配列を決定し、これらの配列を PWV-K, M, S, および TB を含めた既報のポティウイルスと比較した。推定された CP のアミノ酸配列の相同性は、PWV-K, -M, -S, -TB に対し 75~77% であり、PWV-K の -M, -S, -TB に対する相同性 (82~84%) や、PWV-M, -S, -TB 相互の相同性 (95~99%) より低かった。現在容認されつつあるポティウイルスの分類方式すなわち、CP のアミノ酸配列の相同性が 90~99% の場合に同種ウイルス内の別系統とし、38~71% の場合に別種ウイルスとする基準に照らすと、PWV-AO, -K, -TB グループ (-M, -S, -TB) の相互関係は、同種ウイルスとするには類似度が遠いものの、別種ウイルスと結論することはできなかった。また PWV-AO の CP の、SMV-G2 ならびに WMV2 の 2 系統に対する相同性が 75.2, 72.9 および 73.6% であること、さらに PWV-AO の NCR に SMV や WMV2 との共通配列 AG-GTGG-----CCACC が存在することから、PWV は SMV や WMV2 に近縁であることが示唆された。

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