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

Hisashi IWAI**, Junichi SAKAI***, Kaoru HANADA*** and Kei ARAI**

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 μg 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 (Amerham) 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 methods1). 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 (Amerham). 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 (CCCESVSLQ)2,11). 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)14) 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 strains5,18) as well as 6 different potyviruses2-4,11,14,15) 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 soya bean mosaic virus (SMV)-G211) (75.2%) or the strains of watermelon...
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.

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 group$^{19}$ in NCR is underlined with a wavy line.
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

<table>
<thead>
<tr>
<th>Virus</th>
<th>Whole amino acids</th>
<th>Amino acids excluding the N-terminus</th>
<th>3' Non-coding region</th>
<th>Nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV-S</td>
<td>76.5</td>
<td>76.9</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>PWV-TB</td>
<td>76.5</td>
<td>76.9</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>PWV-K</td>
<td>75.6</td>
<td>75.8</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>SMV-G2</td>
<td>75.2</td>
<td>82.4</td>
<td>66.3</td>
<td></td>
</tr>
<tr>
<td>PWV-M</td>
<td>74.8</td>
<td>75.6</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>WMV2-Aus</td>
<td>73.6</td>
<td>84.1</td>
<td>63.6</td>
<td></td>
</tr>
<tr>
<td>WMV2-USA</td>
<td>72.9</td>
<td>84.1</td>
<td>64.4</td>
<td></td>
</tr>
<tr>
<td>SAPV</td>
<td>69.4</td>
<td>77.1</td>
<td>56.5</td>
<td></td>
</tr>
<tr>
<td>PRSV-W</td>
<td>58.1</td>
<td>65.0</td>
<td>50.3</td>
<td></td>
</tr>
<tr>
<td>JGMV</td>
<td>56.2</td>
<td>64.7</td>
<td>nof</td>
<td></td>
</tr>
</tbody>
</table>

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, Quevada et al. (1990); SAPV, Brand et al. (1993); PRSV-W, Quevada 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 virus (SAPV, recently identified as a strain of the cowpea aphid-borne mosaic virus) 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 Ward, 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, 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 NCR may partially support this hypothesis.

**Literature cited**


和 文 摘 要

岩井 久・酒井 淑一・花田 靖・荒井 啓： Passionfruit woodiness virus-瘻美大島株（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-瘻美大島株（PWV-AO）のゲノム RNA の3’末端を含む領域の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 のアミノ酸配列の同定性は、PWV-K、M、S、TB に対して75 ～77%であり、PWV-K の M、S、TB に対する同定性（82 ～84%）や、PWV-M、S、TB 相互の同定性（95～99%）より低かった。現在の種類別にあたるポティウイルスの分類方式ならびに CP のアミノ酸配列の同定性が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たとは類似度が低いものの、別種ウイルスと論ず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系統に対する同定性が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 の近縁であることが示唆された。

(Received December 24, 1996; Accepted September 5, 1997)